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REGENERATION IN PLANTS. II.[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXIX.

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(WITH NINE FIGURES)

WOUND STIMULUS.

THE influence of the wound has been put forward many times, either as the direct cause, or as an important factor in regeneration. WIESNER (**19**) has suggested that between the wounded part and the new structure formed there is a direct causal connection, due to substances developing in the wounded cells and passing to other parts, there inciting the reversion of mature cells into the meristematic condition. GOEBEL (**4**, p. 204) also believes the wound stimulus to be a factor. KLEBS (**9**), on the other hand, rather discredits this idea and thinks that the wound in itself is of no importance in regeneration. The possibility of the wound having a far reaching influence is not at all improbable, for many well-known cases of traumatropism show how cells may be affected at a distance from those actually concerned in the wound. Of the many experiments to determine if such an influence is operating, only a few need be mentioned, and in these cases only the results will be given.

It was seen that with Phaseolus the removal of the stem above the basal primordia is followed by the development of the latter. The removal of the cotyledons, while causing wounds still closer to the young bud, produces no such development; nor does the severing of the stem as close as possible below the primordia. Wounding the stem by cutting notches immediately above the primordia, as

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deep as four-fifths through the stem, has no influence. String was tied tightly around young stems, just above the primordia, and as the stems grew the string cut in deeply on all sides; no results followed. Longitudinal slices, the length of the epicotyl and three-fourths through the stem, produced no results.

Experiment 38.—Notches cut at different points in a spiral around the stem, so that all the bundles were severed, failed to incite the buds below.

Experiment 39.—Five plants 70–100^{cm} high, 6–9 internodes long, and with lower internodes old and hard were used. The tip of each plant was cut off and also the buds from all the nodes below. In three plants the basal primordia produced shoots; one plant died; and the others remained alive, but no shoots formed. Here the wound effect, if there was such, traveled through a distance of nine internodes. A wound, however severe, seems unable to cause the buds to develop if it does not include the complete removal of the growing apex; and even on a large plant the removal of the very tip is all that is necessary. Here, as in the willow and other plants (see experiment 43), the effect of the wound passes only down the stem; a wounding or a complete severance at any point along the stem has no effect on the buds above this point. As will be brought out later, the opposite is true of the roots; that is, the influence of the removal in inciting new roots only passes upwards. This would hardly be true if it were due to the diffusion of substances formed in the wounded cells, as WIESNER supposes. GOEBEL (**5**) has found that in Bryophyllum no wounding at all is needed to produce shoots on the leaves. He encased all the buds on the shoot in plaster so as to prevent further growth, and after a time the buds on the leaves developed. If the leaf blade of Cyclamen is cut off new leafy structures arise along the margins of the petals. But WINKLER (**19**) has shown that this removal is not necessary. He left the blades intact, but incased them in plaster, and soon the leaf-like outgrowths appeared as when the blade was removed. The blade was not injured or wounded in any way by this treatment, though undoubtedly some of its activities were suppressed.

As will be described in experiment 40, I inhibited the growth of the apex of Phaseolus by placing it in a hydrogen atmosphere. The

basal primordia in the air below promptly developed. When the hydrogen was removed, the apex continued growing. Placing the roots in plaster, which inhibited any further growth on their part, also resulted in the development of roots along the stem. In these cases there was no wounding anywhere. It appears that while regeneration follows the removal of certain parts, neither this removal nor the wound incident to it are necessary, since the regeneration occurs equally well when the part is left uninjured and certain of its activities suppressed. The wound, therefore, is not in itself any part of the stimulus.

CORRELATION.

By correlation is meant the influence which one organ or part may exert over another. That the removal of certain parts leads to changes within the plant that may modify markedly the growth or function of other structures is a matter of common observation. Examples of this interdependence among the different members of the plant body are abundant. JOST (**8**) has shown that in Phaseolus the mere presence of the leaf is a necessary condition to the development of the bundles of the leaf trace. PISCHINGER (**16**) determined that if the large cotyledon of Streptocarpus be removed the small functionless one will develop into a large one. According to GOEBEL (**6**, p. 809) the early removal of foliage leaves induces the bud scales to develop into the foliar structures. If the upper end of a Taraxacum root be cut away, removing all the buds, new buds soon arise from the cortex below; this is true of many roots. IRMISCH and others (**10**) have shown in many species that if seedlings be cut off below the first node, new buds arise out of the tissue of the hypocotyl. The removal of the growing tip on many shoots is followed by the development of the dormant axillary buds. As is commonly observed in cultivation, if the lateral roots be destroyed, their place is taken by new roots, which otherwise would not have developed. GOEBEL (**2** and **5**) showed that in Begonia and Bryophyllum, and this is probably true for many other plants, the removal of all the buds on the shoot will result in the development of buds on the leaves.

The process by which such an influence is exerted by one part over another is the main problem to be solved in regeneration. GOEBEL attempts to explain it in one of two ways: either (1) the one

part monopolizes the nutritive material to such an extent that the other parts concerned cannot obtain sufficient to enable growth to go on; or (2) he applies SACH'S *Stoff-form* hypothesis. According to this (**17**), there are formed in the plant small quantities of different substances, presumably of enzyme-like nature, each one having the capacity to incite the formation of a definite structure. These substances are supposed to move in definite directions, and where they accumulate in sufficient quantities start the development of the particular structure they are concerned with. In GOEBEL'S opinion (**4**, p. 204) the influence of external conditions are of little account in regeneration, the important cause being "the direction in which the constructive material moves." GOEBEL says (**3**, p. 42) "the vegetative points act as centers of attraction for the plastic material, their influence being stronger or weaker according to their position." In Bryophyllum, for example, the apex of the shoot is the strongest, then the lateral buds, and last of all the vegetative points on the leaves; so that the apex is able to draw to itself the greater part of the "constructive material;" but if this apex is removed, the lateral buds will be able to "attract" this substance; and in the absence of these lateral buds, the growing points on the leaf are able to appropriate it. In Begonia no growing points are present on the leaf, but when it is removed GOEBEL says bud-forming material accumulates at the base and induces the formation of buds there. If this material, formed in the leaves, moves toward the base of the leaf and passes out because it is "attracted" by the growing points on the stem, just why it should continue to flow in that direction and accumulate at the base, when all connection with these "centers of attraction" is broken, is one of the unexplained difficulties that beset this hypothesis on every hand. MORGAN (**12**) has strongly objected to this theory, but his evidence against it does not seem to me to be necessarily fatal. GOEBEL in a recent paper (**5**) is inclined to lay less stress on it than formerly, asserting that the non-development of the buds on the leaves is due to a checking influence exerted by the buds of the shoot; "but," he adds, "whether we are here dealing with a stimulus transmitted along the conducting system, or whether the building material (*Baustoffe*) flowing in the conducting channels is attracted more strongly by the shoot vegetative points than by those on the leaves remains uncertain."

DRIESCH (1), who has worked on animals more than on plants, recognizes in correlation the dominant factor in regeneration, and claims that the absence (*Nichtmehrvorhandensein*) of the one part is the cause of the development of the other. The plant, according to him, is not influenced in regeneration by external factors, but being sensitive to something lacking endeavors to replace it. Somewhat analogous to this is NOLL'S idea (14) of a body-forming stimulus (*Körperformreizen*), by which he implies that there is an innate impulse in the organism toward a definite form, and when a part is removed the resulting disturbance (*Formstörung*) acts as a stimulus to the reconstruction of the whole.

Such hypotheses as these are at present as incapable of demonstration as they are of refutation, and can only serve a useful purpose if they form the starting point for experimentation. Unfortunately they can scarcely be said to do that. Quite different from these is the view held by KLEBS (9, p. 109), who believes that the removal only serves to bring about those conditions, such as accumulation of moisture, changes of a nutritive nature, etc., which would under all other circumstances cause a similar development. To take a specific case, the normal absence of roots on the stems of Salix, KLEBS says, is due to the retarding influence of light, of dry air, and to the fact that the water is being used by the leaves and young parts. When these conditions are supplanted by those of moist air and abundance of water, the roots develop quite independently of any removal or wounding. The experimental evidence that follows shows, however, that the problem is much more complicated than this.

The experiments described have shown the dependent relation that exists between the growth of the apex and the non-development of the buds below. On Phaseolus the basal primordia do not develop so long as there are buds above them developing. Indeed, only one bud is sufficient for this; for if the upper part be cut away and all the buds but one be removed, the basal buds do not start.

Experiment 40.—The stems were cut off at the second internode, and the buds from one side of the base of this were removed; and in some cases not only the bud but the leaf and one-half the diameter of the epicotyl for its entire length was sliced away. The remaining bud grew vigorously, but neither of the buds at the base developed. If at any time this bud was cut away those at the base promptly started.

Experiment 41.—The buds at all the nodes were removed from a larger number of plants, leaving the growing tip and the basal primordia; the latter did not develop. When the tip also was removed they started promptly, even though they were separated from it by a distance of 70^{cm}—six to nine internodes. *Figure 1* shows one with three internodes.

FIG. 1.—*Phaseolus:* Tip and all upper buds removed; buds at base soon developed shoots.

It has been shown that no amount of wounding short of the complete severance of the stem will produce this result. GOEBEL (**2**, p. 386) says that in Circaea if the central orthotropous shoot be allowed to grow in a dark chamber, it has the same effect on the lateral plagiotropic shoots as if it were removed; that is, one or more of these become orthotropous.

Experiment 42.—Young plants of Phaseolus were taken when the epicotyl was 5 or 6^{cm} long, the first pair of leaves just unfolding and the apex with the leaves directed into a dark chamber. The lower part of the stems were in light and the roots in soil. The parts in the dark elongated rapidly and soon were completely etiolated, but in no case did the buds below develop. Similar experiments with Salix showed this would not cause the axial buds below the part in the dark to develop. It would look as though a complete removal of the apex is necessary to start into activity the latent growing points below, but experiment 43 shows that this is not so, for there the tips of the four young plants were passed into a bell jar and sealed air tight with wax, and through the bell jar a continuous current of hydrogen gas was passed. The growth slowed down, and after about twenty-four hours ceased entirely; and in a few days the buds in the axils of the cotyledons below started to develop and grew quite vigorously. Upon the removal of the bell jar the apices of the shoots continued to grow. This shows that only a cessation of certain

activities, presumably those concerned with growth, is necessary; and it has been shown above that however this may act on the buds below, it is not through any disturbance created in nutritive or water relations.

GOEBEL (**5**) has shown that if in Bryophyllum all the buds on the stem are prevented from growing by encasing them in plaster, the growing points on the leaves develop. HERING (**7**) showed that the small cotyledon of Streptocarpus would develop both structurally and functionally into the large one, if the latter were prevented mechanically—by plaster—from growing. WINKLER (**19**) has found that by a similar treatment of the leaf blade of Cyclamen the new leaf-like structures that develop when the blade is removed will arise from the margin of the petiole. He leaves us in doubt as to whether he selected leaves that had entirely ceased growth before his experiments began. He thinks that the regeneration is due to the interruption of one or more of the functions; either respiration, transpiration, or photosynthesis. My results show that for Phaseolus and Salix cessation of neither transpiration nor photosynthesis will cause regeneration. Respiration is checked in the hydrogen, but what other changes may be involved it is impossible to say.

On those plants whose growing tip soon ceases activity and dies, as *Syringa vulgaris*, I have not been able to induce the axillary buds to develop by removing the apical part of the shoot. In all of many cases tried, however, in which the terminal growing points continue their activity during the growing period, their early removal was followed by the sprouting of latent buds below (*fig. 2*). This is true even in those plants whose annual shoots are without branches, and in the axils of whose leaves the buds cannot be seen even with the aid of a lens.

Lack of space prohibits a detailed description of these experiments, and only the results need be given. In the majority of cases it is the buds near the apex that start (*fig. 3*), but occasionally almost every one on the shoot starts; and in one case, on a shoot of Salix, only those at the base started. The young shoots were either cut off and the base placed in water, or else they were left attached to the plant. If the tip is cut off and also all the leaves, the buds develop. Or if the tip is removed and the shoot placed in the moist

chambers mentioned above, so that transpiration is entirely prevented, they also develop. In these moist chambers, however, with the tips still intact, no development of the buds occurs. If the shoot is removed and placed in darkness for a few days, until the food is mostly exhausted by the rapid growth, and then the apex cut off, the lateral buds still develop. The removal of the leaves has no influence on the buds, for from many shoots not only were all the larger leaves carefully cut away, but even those still folded in the buds, and in no case did the growing points develop. But when in addition the tip of the shoot was removed, they at once started. While these experiments were mostly on Salix, other plants, such as Cornus, Lycium, Ficus, Oleander, etc., gave similar results. Here, as in the basal primordia of Phaseolus, no matter how vigorous and well-nourished the plant may be, or how abundant its water supply, with the growing apex of the shoot intact, the young axillary buds remained dormant. When this tip is removed, the bud starts to develop even with a loss of water or in a starved condition. This capacity is greatest in the young shoot, and gradually declines with age as the seasonal growth ceases. In

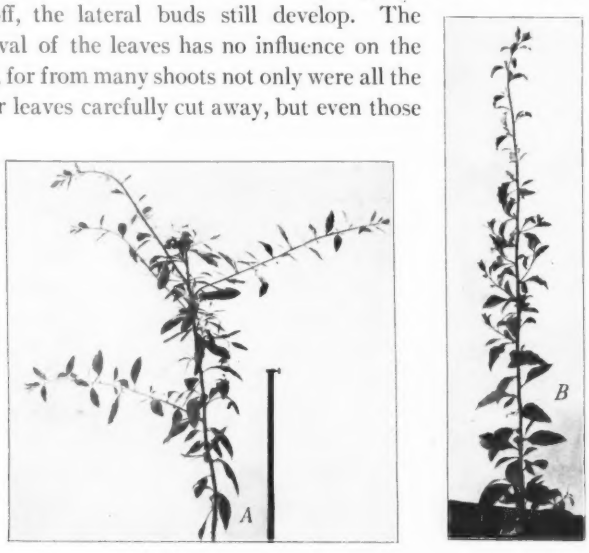

FIG. 2.—*Lycium halimifolium*. Two similar shoots were selected and from *A* the apex was removed; the photographs show them both three weeks later.

annuals, like Helianthus, whose axillary buds finally develop branches, the removal of the apex from young plants causes the buds to develop at once. *Silphium integrifolium* has a leafy stem, unbranched until late in the season when the flower branches arise; but if the apex is early removed, the minute axillary buds promptly produce branches.

FIG. 3.—Young shoot of *Salix amygdaloides*. The apex was cut off at *a* and four branches developed below this.

Not only does the growing apex exert an influence felt by the growing points below it, but those along the shoot exert a similar influence upon those lower down. VÖCHTING (**18**) showed that in isolated pieces of Salix stem only the buds toward the upper end of the piece develop. PFEFFER (**15**) found that if the upper buds are placed in plaster those lower down start. As a series of experiments on polarity will be published in an article to follow this one, only brief mention will be made here of the experiments in this connection. The plant used was Salix.

Experiment 43.—Four pieces of two-year old stem, 35^{cm} long, were placed with the basal ends in 6^{cm} of water. Two were erect, with the remaining parts in the moist air; the upper 8^{cm} of the other two were in a bell jar through which a current of hydrogen passed. In a few days the buds at the upper part of the first two started to develop; and, so far as could be observed, simultaneously with them those on the other two pieces just below the part in hydrogen. Those in hydrogen were not killed, and when the gas was removed started to grow. In a few weeks their shoots had surpassed those below them, which had now almost ceased growing.

Experiment 44.—Twelve similar pieces of stem were selected, 30^{cm} long. On three the buds were left only on the upper third;

on three more only on the middle third; on three more only on the lower third; while all the buds were left on the last three. These were all placed horizontal in moist air. On the last three mentioned only the upper buds formed shoots; and at the same time most of the buds, especially the uppermost ones, started on all the other pieces. Many tests showed that any bud at any place along the stem would develop if the buds above it were prevented from doing so. Here again, it is not a question of nutrition or water, for the stems and buds are filled with reserve food, and in a constant spray (in which the experiment was repeated) there can be no lack of water.

Whatever the influence of the growing buds may be, it is felt only on those below them, and not on the buds above them (that is, toward the apex of the axis). In the experiment just mentioned, in which the upper buds were inhibited by hydrogen, those below had gotten a good start and were forming shoots, but when the hydrogen was removed, the upper ones developed as usual.

Experiment 45.—A piece of Salix stem, 30^{cm} long, was placed so that the 12^{cm} in the center was in a continuous spray, and the two ends in quite dry air. The buds in the central portion swelled up and burst open before the others showed any signs of swelling. The whole piece was then placed horizontal in moist air. The buds at the apical end soon enlarged and developed shoots; but those at the lower part did not. These central buds, while able to prevent the buds below them from developing, had no influence on those above them. Finally, all the young shoots and buds were cut off from the upper two-thirds of the piece, and the upper ones on the basal third promptly started.

If the entire piece is surrounded by the same conditions, the shoots all appear at the apical end; but by placing the basal end in water and the rest in dry air, the buds in the water or close to the surface start first; but soon the upper ones commence to grow, apparently indifferent to those below them; and as the young shoots increase in size, those below become less vigorous and are often finally suppressed. Other experiments of a kindred nature might be mentioned, but these will suffice to indicate that the development of the buds at any region along the stem tends to suppress those

below them from developing, but does not influence those above them.

Here we are dealing with growing points already laid down, but the same principle holds where these do not exist. If we place a root of Taraxacum in a moist condition, the buds at the top will soon develop; but if we remove all these buds, entirely new ones will be organized and develop. If these be prevented from growing, lower down others will be organized, as GOEBEL has shown (**2**, p. 492). Here the organization of new shoot primordia along the root does not occur so long as those at the top are allowed to grow. Here again we cannot attribute this to the monopolizing of the food or water by the upper part, for these are abundant everywhere. There is a direct relation between the growth of the shoots at the top and the non-formation of buds lower down, entirely independent of these two factors.

The development of new roots when those present are removed shows a similar phenomenon. The behavior of Phaseolus in this connection may be briefly mentioned. Plants grown with roots in water cultures developed a vigorous root system. From some of these all the lateral roots were removed, leaving only the main root. Soon numerous new lateral roots arose and grew vigorously; these were cut off and still others came on, though not so vigorously. In the mean time no new lateral roots had come out among the older ones on those that had not been so treated. If we cut off the main root transversely, numerous roots arise just above the cut; and if we cut away the whole root system by severing the stem at the base, new roots arise on the lower part of the stem. Sometimes the roots are so numerous here that I have counted eighty-one coming out of the lowest centimeter of a Phaseolus stem less than 5^{mm} in diameter. It was found almost impossible to produce roots on any part of the stem that is in direct connection with roots below, but when this connection is broken roots promptly start.

Experiment 46.—Local regions of several stems were surrounded by water in glass cylinders, as in *fig. 4*. On some the roots were intact, and from others the stem was cut off at the base. The roots and the lower ends of the stems were in water. On those with roots still attached no roots formed on the part of the stem surrounded by

water; but the others produced abundant roots there and also at the lower end (*fig. 5*). Finally, from one of the former the root system was removed by severing the stem at the base, and vigorous roots then appeared in the cylinder, as well as on the lower end of the stem.

The influence exerted by the roots very evidently passes along the vascular bundles, for the transverse cutting of these at any place leads to the origin of roots just above the cut. The cutting of the cortex as deep as the bundles has no results. If a notch be cut in the stem so as to sever some of the bundles, roots appear only above, not below the notch (*fig. 6*). If the stem is cut off near the base, roots come at this place; but if it be cut off further up, the roots come there even more vigorously. If a series of notches, either one

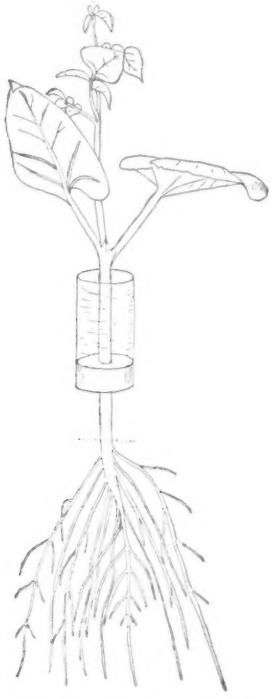

FIG. 4.—*Phaseolus:*—A short glass tube attached around stem and filled with water. Roots, grown in water culture, left uninjured and submerged. No roots formed on stem within tube.

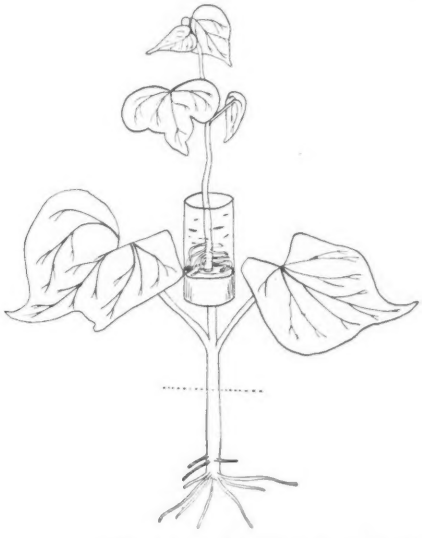

FIG 5.—*Phaseolus:* Entire root system removed; otherwise as in *fig. 4*. Roots develop both in tube and in water at base of stem.

directly above the other or on different sides, be cut, roots come from above each of them, but more vigorously from the upper ones, due probably to that part being nearer the source of food supply. Or when stems are cut through, some at the base and some higher up, the roots appear perceptibly sooner on the latter. No matter where the stem is cut off, roots develop immediately above this point, showing that the pericycle has the power to produce roots at any point. Yet, as has been stated, if the stem be cut off, say near the base, roots come only here, though the whole stem is submerged in water. But when the cut ends of the stems were encased in plaster so as to prevent roots from coming there, they came further up. Also stems whose lower internodes were 10–12^{cm} long were placed in water, and every day 0.5^{cm} was cut off. In ten days roots appeared scattered along the remaining part.

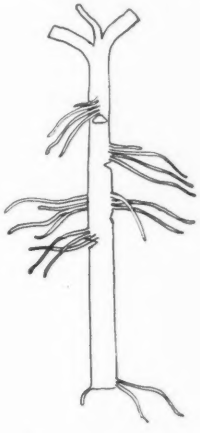

FIG. 6.—*Phaseolus:* Lower internode of stem notched on different sides and completely submerged. Roots appear only above the notches.

Experiment 47.—Portions of stems with roots intact were surrounded by glass cylinders 4–5^{cm} long and made air-tight at each end by means of rubber stoppers and wax, and opening

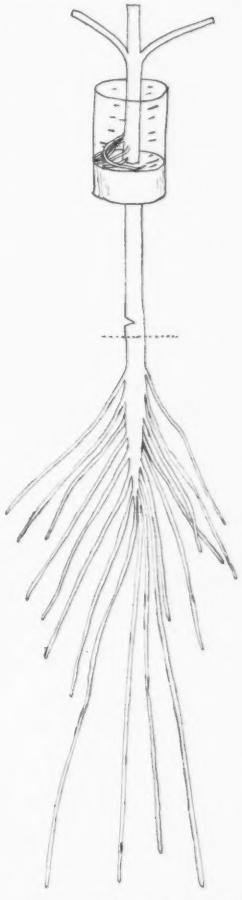

FIG. 7.—*Phaseolus:* Part of stem surrounded by water as in *fig. 4*. Roots intact and submerged to dotted line above which stem is notched to center. Roots appeared in tube, arising only on side directly above notch.

into a vessel containing a 3 per cent. solution of ether. This seemed to anæsthetize the stem without killing it, and roots appeared just above this portion. The effect of the anæsthetic probably was to prevent any passage of stimuli through this part of the stem either up or down. Experiment 30 is instructive in this connection. It will be recalled that surrounding the stem at any place with water in glass cylinders will not start roots at that place if the connection with the roots below is unbroken; but, as shown in *fig.* 7, if a notch is cut in the stem some distance below, thus severing connection with the roots, roots will appear above the notch in the water, apparently coming from those bundles severed by the notch. In other words, the water supplies a favorable condition for root development, and the cells are able to act as soon as the connection with the roots below is broken. If the air be moist, roots come out also immediately above the notch.

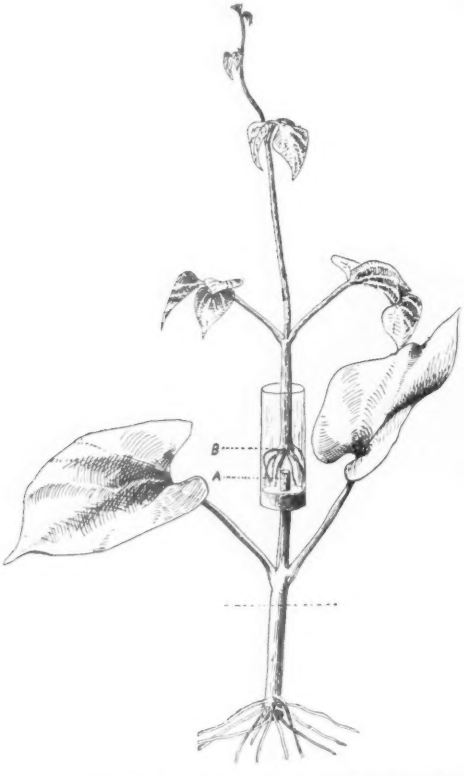

FIG. 8.—*Phaseolus:* Stem cut off at base; lower end submerged. Portion of next internode above surrounded by water as in *fig.* 4, and stem severed; *A*, *B*, ends slightly separated. Roots appeared only at *B*.

When a stem is arranged as in *fig.* 8, roots always arise on the part just above the cut, never on the part just below it, even though this piece is inverted with its end in water, as in *fig.* 9. This difference in the behavior of the cells

at the two places cannot be due to any difference in the amount of water or nutritive material available at the two places, for both may be saturated with water and equally well provided with food. Nor can it be that the cells just below the cut have any less capacity for root production than those just above; for if the cut be made a little lower down, so that the cells which formed the upper part of the lower piece now form the lower part of the upper piece, they promptly produce roots. It is evident that there is some factor operating on the cells at the one point that is not present at the other; and it seems equally evident that this is not a condition of moisture, of nutrition, or of a wound influence, for all of these are equal in each case. As stated above, when no cut is made there is no tendency for any of these cells to form roots; but as soon as an incision is made, there is a change in the behavior of the cells above it, while those below remain uninfluenced. In the present state of our knowledge there seem only two possible lines of explanation: (1) as a result of the incision, new conditions are added to the cells above the cut which serve as a stimulus inciting them to root production, conditions not acting on the cells just below the cut; or (2) the cells above the cut have been relieved of some influence that previously prevented their growth, an influence still acting on the cells below the incision, and which acts on all the cells when no incision is made.

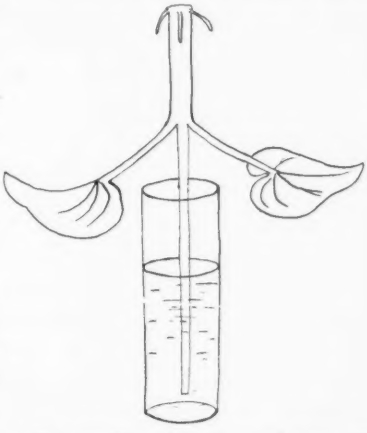

FIG. 9.—*Phaseolus:* Inverted stem with first pair of leaves and adjacent parts of internodes. Apex in water, base in moist air. Roots arising only on base.

Recurring to the first idea for a moment, we have noticed that there are two different conceptions current. The one is that these new conditions added are the results of changes induced by such factors as nutrition, moisture, wounding, light, gravity, aeration, etc. While light retards the development of roots in this case, it

does not modify their distribution; and as the roots occur in the same way whether the piece is erect, horizontal, or inverted, gravity cannot be a determining factor. The other conception is that of specific formative substances, which in this case, moving toward the base, would accumulate at the lowest part and incite the formation of roots there. As this theory is to be discussed later, only two cases will be mentioned here to show its inefficiency. If in Salix, instead of cutting the stem off just above a bud, the bud is cut entirely away from the stem, it starts to develop just the same; or, as in *fig. 7*, where a notch is cut at the base of the stem, there can be no accumulation of these substances further up where the roots actually occur. It is not impossible that the upper parts, especially the leaves, exert some influence on the formation of roots on the stem. If so, it is not through the transpiration current, for the roots develop as well when transpiration is entirely checked as when it is quite active. Also, in *fig. 9* the leaves are transpiring and a current must be passing in through the end that does not produce roots. The removal of all leaves greatly decreases the vigor of the roots and also the number that are formed, but the same result is obtained by placing the leafy part in the dark, or in an atmosphere free from carbon dioxid. The fact that pieces of the internode produce roots indicates that the leaves are not necessary; their influence probably lies in keeping the stem better nourished.

On the other hand, the evidence seems to point to the second line of explanation, namely, that just as the growing shoots seem to exert a retarding influence on the buds below them, so the growing roots exert an influence which inhibits cells, otherwise able to do so, from forming roots. If, as just mentioned, the stem is cut off at its lower end and placed in water, and at the same time a portion of the stem higher up is surrounded with water, we get roots at both places. When these are growing well, if we cut off the lower part of the stem having the roots on and then submerge this end again, new roots soon appear at this point, although roots are growing vigorously a few centimeters above. In no case could I ever get a retarding influence of the roots to pass down the stem.

The problem to be solved in regeneration seems to be not so much the growth of parts following a removal (the causes here are

those that induce growth everywhere), but the cause of non-development in the normal life of the plant; and this seems to lie in that influence which one part may exert over other parts or throughout the entire plant. A glance will show how universal this is among plants and the variety of ways in which it may manifest itself. If the main shoot of spruce is cut off, one or perhaps more of the dorsiventral plagiotropic lateral shoots will change their nature and become erect and radial. Many bulbous and tuberous plants do not produce seeds normally, but if the bulbs or tubers are removed seed production is then accomplished. This cannot be explained on the ground of specific bulb and seed forming substances, since if we assume the existence of these two substances, we must assume them to be different. GOEBEL (**3**, p. 213) says "in the normal condition the seed formation is hindered because the plastic material which might be used for the seeds streams into the bulb, where it is turned to account in the formation of bulbils for asexual reproduction." The assumption is that the nutritive materials, streaming to the point where the bulbs or tubers are to be formed, incite the formation of these organs; and if these are prevented from forming, the material will flow toward the flowers and there stimulate seed formation. This supposition is exactly the opposite of what actually occurs in plants. The nutritive, or any other soluble material, diffuses from its point of greatest density in all directions, as well toward the seed as toward the bulbs, and it will diffuse in one direction rather than the other because it is there being either changed or removed from solution by the activity of the cells. The "streaming" does not start the growth, but the growth activities remove the material from solution, and diffusion is set up in that direction due to the lowering of the concentration at that place. Growth or other cell activities involving a use or change of material must of necessity precede any movement other than diffusion in all directions. We have no reason for assuming that the food made in the leaves would diffuse toward the bulb any faster than toward the seed, and with the whole plant in a well-nourished condition it is scarcely possible that the amount available would be so slight that the embryos would still be in a condition of starvation so extreme that they could not even start to grow. If the latter were true, the diffusion of food

materials from the leaves would be stronger toward this point than toward the tuber of any other point in the plant. There seems to be some factor dependent on the presence of growing tubers or bulbs which prevents the fertilized embryos from developing even in the presence of sufficient food and moisture. Kindred phenomena are common, *e. g.*, the death of the fern prothallium with the developing of the embryo. This cannot be due primarily to starvation, since each cell retains its own mechanism for food manufacture.

MORGAN (**11**, p. 272) has suggested that these phenomena are due to differences of tension existing throughout the plastic parts of the plants. "As long as the apical bud is present at the end of the stem or branch, or even near the apex, it exerts a pull or tension that holds the development of the parts in check; but if the apical bud is removed, the tension is relaxed and the chance for another bud developing is given." And further, MORGAN suggests that "from the apex of the plant to its base the tension is graded, being least at the apex and increasing as we pass to the base," so that, when the apex is removed, "those buds will develop first that are on the region of least tension, and then development will hold in check the other buds by increasing or establishing the tension on the lower part of the piece." Just what this "tension" may be is not very clear, and with GOEBEL I am unable to see that it makes the matter any plainer. MORGAN has suggested later (**12**) that if this idea of differences in tension is too vague, it can be given a more practical form by assuming it to be the outcome of osmotic differences in the cells. Diligent search has failed to reveal these in Salix or Phaseolus, and there seems to be no basis for the assumption.

MORGAN has more recently modified this hypothesis by another suggestion (**13**), according to which the difference in the development of buds at the two ends of a piece of stem is due to the relative state of development of the buds. In the willow, for example, those toward the apical end have reached a greater degree of maturity than those lower down, and so naturally are the first to develop. A few experiments will show this hypothesis to be quite untenable. Experiments already mentioned have indicated that when we take two pieces approximately alike, and remove all except the basal buds from one, these buds will develop simultaneously with the

apical buds of the other. Indeed if any bud be selected, and the stem cut off just above it, the bud develops; but if the stem be cut off just below this same bud, so as to leave it at the base of the piece, it will not develop. Two pieces of Salix stem were selected as nearly alike as possible, and of exactly the same age. At about the center of each several buds, all alike, were selected; and each piece was cut in two, one so that these buds remained at the apical end, and the other so that they were at the basal end. In the former they developed and in the latter they did not. Experiments could be multiplied indefinitely to show that all the buds along the stem are equally able to develop; and whether any particular one does or not depends on whether the piece be cut so as to leave it near the apical or basal end.

The development of any plant involves the growth of a few and the suppression of many potential structures; and this is true not only of the vegetative buds, but also of other parts. In the ovary of Tilia, for example, ten ovules are present and may all be fertilized; but very soon nine cease activities and one only continues to form an embryo. A similar event occurs in Pinus and other plants. Were it merely a question of food, a fierce struggle would ensue among the developing embryos, and some at least would continue for a long time in a more or less starved condition. In Pinus practically all the embryos except one stop growing, while all about them are disintegrating tissues liberating food materials, some of which must pass by, or even through the arrested embryos to get to the one that continues growing. The formation of the embryo in many plants is accompanied—or immediately followed—by the development of other parts, often more or less distant, *e. g.*, the large fruit of the melon, an event that we cannot attribute to any increase of nutrition resulting from the developing of the embryo.

In addition to the growth at the meristematic growing points and along special regions which remain meristematic, as the cambium, the capacity for vegetative development is retained by many of the differentiated tissues in various parts of the plant body. That is, as the cells of the embryonic tissue differentiate into other forms and assume other functions they may still remain embryonic, in the sense of retaining complete reproductive capacity. So we find

often matured cells of the leaf or cortex quite as able to form new organs as the cells of the meristematic apex of the shoot. In many cases, as *Tolmiea Menziesii*, *Cardamine pratensis*, *Asplenium bulbiferum*, *Camptosorus rhizophyllus*, in the ordinary course of development vegetative growing points arise on the leaves as well as on the shoot, and produce new members in the same way. As a rule, the more luxuriant the growth the more of these buds will be organized and develop; but usually, as in Tolmiea, even under the most favorable conditions not all the leaves on a plant will produce shoots. But I have found in this plant that every leaf produces a shoot when separated from the plant. In *Bryophyllum crenatum*, there are numerous growing points along the margin of the leaf which do not usually develop further. They bear a similar relation to the growing points of the shoot as do the young axial buds of Salix to the growing point; for when the influence of these shoot buds is removed, those on the leaf form shoots.

On the other hand, in such leaves as Begonia the cells do not start to exercise this reproductive power by organizing growing points so long as they are in connection with other growing points of the plants; and GOEBEL showed that in Begonia, upon the removal of all the growing points of the stem, the leaf will organize them.

The same principle holds for other parts. In many roots the capacity for shoot development is expressed in the formation of "suckers," as in willows and other trees; but in other plants, probably the majority, as in Taraxacum, this ability seems able to express itself only when the influence of the shoot above has been removed.

Protoplasmic continuity from cell to cell throughout the entire living plant may fairly be accepted as demonstrated and the existence of various stimuli, either accelerating or retarding, emanating from different masses of tissue and affecting other even somewhat remote tissues is not at all impossible. Indeed, such a transmission of stimuli necessarily occurs in many of the tropisms, where the receptive region is separated by some distance from the region of response. The whole development of the plant body necessarily involves the suppression of many and the development of relatively few, either actual or potential, primordia; and the means by which this is accomplished (correlation if we must have a name) underlies

in a most fundamental manner the entire organization of the plant. All the meristematic tissue and in many cases much of the differentiated tissue contains various potentialities of growth, potentialities which seem impossible of expression while in organic connection with certain growing parts. This interdependence of parts may be manifested in an inhibiting influence, as in the case of the roots or shoots mentioned, or in an accelerating effect, as in the growth of the fruit and adjoining parts after fertilization, or perhaps more correctly with the developing of the embryo. The experiments described indicate that the means of accomplishing this, that is the means by which, for example, a terminal bud suppresses the development of the other growing points on the stem or leaf, do not lie in the withdrawal by the former of the nutritive materials or the water. The theory of specific formative materials fails to account for it; nor does the tension hypothesis add anything to our knowledge of the process. Correlation, the endeavor of the plant to replace something lacking (DRIESCH), and form-stimulus (*Körperformreizen* of NOLL) are statements of the phenomenon and not at all explanations.

Protoplasmic stimuli emanating from various parts, reaching often throughout the entire organism, and affecting the behavior of the protoplasm of even remote portions are quite conceivable; so also are the formation and diffusion of ferments controlling growth; but we have yet no evidence of the existence of either.

SUMMARY.

A brief summary of the general conclusions thus far may be made as follows: The occurrence of regeneration in plants usually involves the replacement of parts removed, but the same result is often obtained when the organ is not removed, but is prevented from functioning. It is often inseparable from the ordinary growth of the plant, as for example when buds arise on the leaves of Tolmiea or Cardamine in ordinary course of the vegetative development of these plants; and the causes here are, no doubt, not different from those that induce the origin of buds on the growing points of the stem. The plant possesses innumerable growing points either organized or potential, the vast majority of which must not be allowed to develop

if the plant body is to retain anything like a definite organization. In most cases this development does not occur in the ordinary life of the plant, because these cells, capable of producing new organs, are held in check by those parts already growing. This non-development does not seem to be due to any lack of those conditions that favor growth, as nutrition and moisture; or to such influences as light and gravity; or to a lack of definite "formative substance;" but to some influence independent of all these, which an organ, acting perhaps along the protoplasmic connections, is able to exert over other parts and so prevent their growth. When this influence is removed, the favorable growth condition, present all the time, permits the growth of the part to occur. In such a controlling influence of growing organs over the numerous potential growing points throughout the plant there exists very evidently a principle of fundamental importance in plant organization.

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A BOTANICAL SURVEY OF THE HURON RIVER VALLEY.

III. THE PLANT SOCIETIES OF THE BAYOU AT YPSILANTI, MICHIGAN.

FORREST B. H. BROWN.

(WITH MAP AND FIVE FIGURES)

INTRODUCTION.

THE materials for the present paper were collected from a detailed survey of an area of sixty acres, known as the "bayou," which lies to the west and southwest of the Ypsilanti Highland Cemetery. It includes steep slopes, a large bayou, with a stretch of floodplain and stream, embracing a wide variety of conditions. From a reconnaissance of the entire course of the stream, it was found that many of the ecological conditions occurring from the source to the mouth were represented at the bayou area. In this small territory, the plant societies have reached an unmolested development, exceptionally favorable for their study.

The results here presented are the outcome of an attempt (1) to determine what plant societies are clearly represented, paying special attention to those which are found also in other parts of the stream course; (2) to determine so far as possible, from existing evidence, the influence of the factors of the environment which, both past and present, may account for their presence or explain their origin. Naturally the stream course societies are considered, leading to a different line of study from the two previous papers of this series, in which the glacial lakes included within the valley have been made the subject of treatment. A careful record of all species found upon the area was kept and a complete herbarium made, which is to be left at the herbarium of the University of Michigan. The work was carried on under the direction of Professor V. M. SPALDING, and the writer desires to express his indebtedness to him for many helpful suggestions.

DESCRIPTION.

1. TOPOGRAPHY AND PHYSICAL GEOGRAPHY.—In the vicinity of Ann Arbor, Ypsilanti, and other parts of its lower course, the Huron River flows through a wide valley with flats bordered by bluffs, often

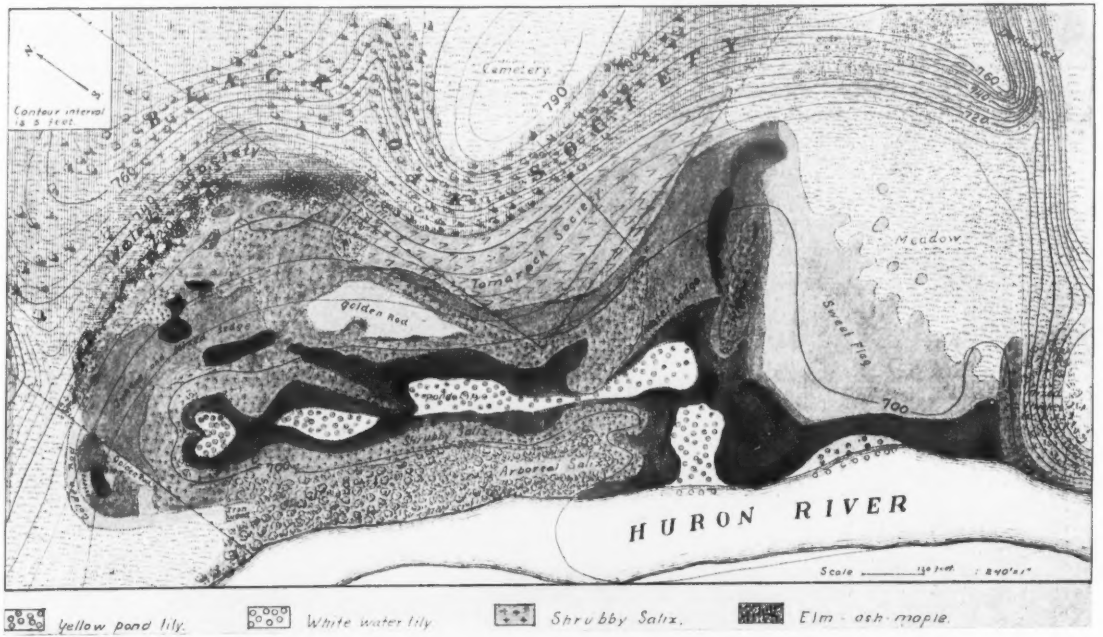
very steep, which rise to a height of some 90 feet above the water. The portion mapped is a fair type of the portion between Ypsilanti and Rawsonville, a village four miles southeast of the bayou. Throughout this part of its course the stream makes a number of loops and bends that would cause a boat to journey over ten miles to reach a linear distance of four.

2. GEOLOGICAL HISTORY. —The cemetery bluff, shown in the upper middle part of the *map* (p. 266) and also in *fig. 1*, is a portion of an old sand bar, "which trends north about a mile, and connects with a beach leading in from the west, which forms the continuation of the western or main ridge found south of the Huron River."[1] This beach is one of two marking the northwest shore of Lake Maumee, which was the first of a series of large glacial lakes formed in this region with the retreat of the glacial ice. Later a change of outlet permitted the water to subside, forming Lake Whittlesey at a level some 75 feet below the upper Maumee beach. During its existence this lake formed the well defined Belmore beach, meeting the river about three miles southeast of the bayou. Still farther subsidence of the waters brought about the formation of Lake Warren, by whose waves the Forest beaches were made at levels of 60 to 90 feet below the Belmore, and at a distance of more than seven miles further down stream. From this point there is a stretch of fixed sand dunes extending to within eight miles of Lake Erie.

FIG. 1.—The cemetery bluff, shown in the upper middle part of map; a portion of an ancient sand bar.

During the Lake Maumee stages, ice and water had not yet uncovered the bayou area, except for high points of the sand bar. During the Lake Whittlesey stage, the stream was cutting its first

[1] LEVERETT, F., Glacial formations and drainage features of the Erie and Ohio Basins. U. S. Geol. Survey **41**:chaps. 14–16.



Map of bayou showing distribution of societies in relation to water and topography; scale 240 feet to the inch.

bluffs. As the waters had now subsided only about 50 feet, the base of the cemetery bluff was as yet under water; but the part exposed was free for the first vegetation to come in. It was not until the Lake Warren stage that the river, through the fall of waters at its mouth, cut still deeper in its channel and exposed the base of the cemetery bluff. The land area which was for a time submerged

FIG. 2.—*Carex stricta*, the deepest water sedge here, appears on the advance of the line of sedges, and forms tufts of great compactness and strength.

and then exposed by degrees forms the large relatively level portion of southeast Michigan, extending for thirty miles or more west from Lake Erie and the Detroit River, and nearly the same distance southward into Ohio. Westward from the basin area the surface is broken up into countless hills, ridges, and kettle-holes, characteristic of the morainal regions. It has been known for some time that the vegetation in this portion of the Huron-Erie basin is distinct from that of the morainal area, with a line of tension in sight of the east limits of the city of Ypsilanti; but an explanation of this has not yet been offered, and does not fall within the limits of the work in hand.

3. THE SOILS.—The distribution of surface soils is important because of the relations existing between soil types and plant societies. The floodplain, while having areas of peaty earth about the center and between the ponds and bluff, with an outwash of sand at the northwest and southeast, is fundamentally of compact till, which underlies the whole region and frequently appears elsewhere at the surface. The east bluffs are more porous, being composed of gravel or sand. A long ridge of mineral soil reaches from the northwest, forming a low barrier along the stream to the marsh about the bay, where it is bordered by peat-like earth. Humus is present everywhere except on the washed slopes of the bluffs. At the east is a line 600 feet in length, following the base of the cemetery bluff, where sand borders the till below with the sharpness of exposed strata. A profile was made through *B* (*map*), shown in *fig. 3*, which further explains this peculiarity in the structure of the bluff. The sand or gravel-capped bluff, with seepage springs along its soil line, and with its structure plainly revealed by the plant societies which inhabit its slope, occurs commonly in the valley.

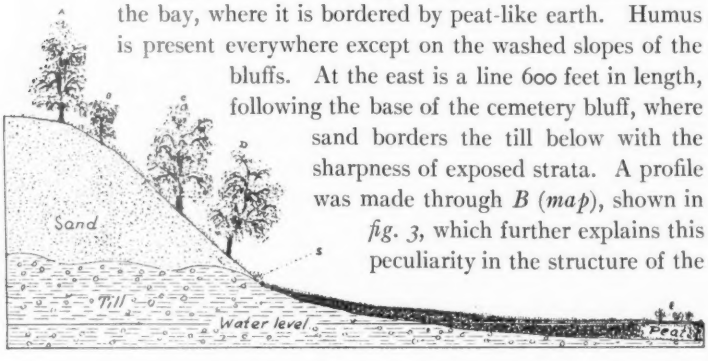


FIG. 3.—Profile through *B*, map, showing influence of plant associations in preventing the outwash of soil, and in the formation of beds of peat; *A*, black oak; *B*, black locust; *C*, yellow oak (*Quercus acuminata*); *D*, bur oak; *S*, seepage springs; *E*, shrubs (*Betula pumila*); scale 60 feet to the inch.

4. SOIL WATER.—The conditions of soil moisture, as influenced by varying soils and differences of elevation, have an obvious present bearing upon the distribution of plant societies. Conditions of extreme dryness are to be found in places along the brink of the steeper bluffs, where the combined effects are felt from the porous nature of the soil, elevation, and exposure to drying effects of wind and heat. More soil moisture is found on descending the slopes, and the change may be abrupt along soil lines where seepage springs occur. There is a slow but constant flow and percolation of spring

water from the base of the bluffs to the central ponds, giving rise to one of the main differences between the conditions existing in the bayou and the usual kettle-hole swamp or bog in which water is more or less standing. The ridge along the west is usually free from surface water, although always moist.

THE PLANT SOCIETIES.

The vegetation of the bayou and bluffs consists of a number of plant societies, which may be treated under three heads.

1. PEAT-FORMING SOCIETIES.—Under this head are included the societies commencing with the deepest submerged vegetation and reaching landward as far as the elms, red and green ash, and silver maple of the floodplain. A connected account of each in its order is given because the vertical succession of societies which occurs here seems to have been a common one on the wide floodplain areas below Ypsilanti, and would appear to possess some unusual features in the light of what has been written regarding the floodplains of other river valleys. This group comprises 131 species, or 32 per cent. of all species found upon the area surveyed. Seven distinct societies may be distinguished in order as follows, the number of species being given in parenthesis: 1, pondweed (7); 2, waterlily (5); 3, water smartweed (6); 4, Typha-Sparganium (10); 5, water sedge (19); 6, willow (71); 7, tamarack (13); total 131 species.

The first society is submerged, consisting chiefly of species of Potamogeton, and occurs only in the stream. The growth offers resistance to the flow of water, checking the current, and causes deposit of suspended particles in addition to that arising from vegetable decay. Following upon the submerged society, there appears one of floating plants, which still further checks the current. The waterlilies secure a firm anchorage. The white waterlily (*Castalia odorata*) is more common in the current. The yellow pond lily (*Nymphaea advena*) commences just back of this and occupies the bays where the current moves more slowly or is scarcely perceptible. The exchange of species in relation to habitat is best shown between the bay and the current (*map*). With the presence of the waterlily society, there is a marked tendency to secure room near the water surface. The potamogetons appear to rely on the anchorage offered

by the waterlilies, sending out long stems with leaves clustered above. In this zone, when the surface of the water is quiet, occur such plants as Utricularia, Lemna, Spirodela, and Riccia. Peat-like material rapidly accumulates below as growth proceeds above. The interwoven rootstocks are firm enough to support the weight of a person. The several kinds of water smartweed are most active in forming this mat, although the yellow pond lilies add a good deal of firmness.

At length the intervening space between the surface growth above and the substratum below becomes filled in with an oozy accumulation of peat, and gradually two marsh societies appear, the Typha-Sparganium and the water sedge. These marsh plants are characterized by the possession of thickly matted root systems and rhizomes. *Carex stricta* is the deepest water sedge here. It appears on the advance of the line of sedges and forms tufts of great compactness and strength. These tufts persist through subsequent changes that occur, and long after the death of the sedge itself, finally become covered with *Potentilla fruticosa* and turf-forming grasses. They give rise to the peculiar mound or hummock configuration so characteristic of the numerous valley meadows which have been built up in this way. Sedges build up the peat bed in two ways: (1) by the death of the aerial parts; and (2) by the death of the roots and rootstocks, the latter often the more rapid of the two processes.

As the surface becomes rather free from water and firm, there appear the first woody types. Shrubby species of Salix are the first to grow in abundance, covering many parts so densely that other forms are almost excluded. *Rhus venenata*, *Naumburgia thyrsiflora*, *Asclepias incarnata*, and *Scutellaria lateriflora* act as pioneers in seizing decaying timbers, islands of sedge, and similar places of advantage. This results in the formation of a willow or drained swamp society, the vegetation of which is richer in individuals and species than that of any other association. From the map it will be seen that the arboreal species of Salix are confined chiefly to the ridge, which is mainly till in this portion. The shrubby kinds, on the other hand, prevail over the beds of black peat bordering the water.

Part of the drained swamp area has culminated in tamarack and other plants with xerophytic characters similar to those characteristic

of the flora of the so-called undrained swamps. This stage does not appear to have been uncommon in the wider parts of the valley, and possesses points of unusual interest. This association forms a crescent-shaped area at the base of the cemetery bluff, commencing with the seepage springs (*fig. 2* and *map*) and reaching stream-ward as far as the water sedge. It is here 700 feet long and 200 feet wide, containing the following species listed in order of their relative abundance: *Scirpus americanus, Carex filiformis, Koellia flexuosa, Lysimachia quadrifolia, Campanula aparinoides, Lobelia Kalmii, Aster junceus, Parnassia caroliniana, Rynchospora alba, Gerardia paupercula, Solidago Riddellii, Sarracenia purpurea, Larex laricina* (once abundant, but now removed for firewood).

On comparing the composition of this society with that of others, it was found that the above species are peculiar to the one society and occur rarely if at all outside of it. A sharp line of separation occurs between it and neighboring societies. As a whole it may be said that the vegetation is the most distinct of all those studied. With but two exceptions all species are perennials, and a certain likeness of form characterizes the members, as if mutually adapted to xerophytic conditions, causing the vegetation to stand out in contrast with the neighboring broad-leaved hydrophytes. Regarding this group of plant societies the following points are to be noted.

1. Of the whole group, 5 per cent. of the species were found to be submerged, 32 per cent. amphibious, and 63 per cent. terrestrial. The willow society is richest in species, containing more than twice the number of species found in any other society of this group, and slightly more than any society to be discussed later.

2. Of the agencies active in filling the bayou, the first place is assigned to vegetation (*fig. 3*). Not more than one-third the material entering into the composition of earth so formed is inorganic matter, the proportion often being smaller. From the areas of organic soil frequently occurring along the flats it is plain that the process has been as active in past as in present times, converting numerous standing pools and deserted channels into dry land surface supporting a thrifty forest growth.

3. Most of the common species are gregarious, and tension lines are sharply marked.

4. The vertical succession of societies which build up the drained swamp may culminate in tamarack and other plants with xerophytic adaptations.

5. The bayou vegetation is similar to that of like areas along the stream, and only minor differences were brought out; but on comparing this vegetation with that of the glacial lakes about Ann Arbor, the most pronounced differences were discovered when the respective swamp and bog societies were compared. *Andromeda polifolia*, *Chamaedaphne calyculata*, *Arethusa bulbosa*, *Sarracenia purpurea*, *Oxycoccus macrocarpus*, *Drosera rotundifolia*, and sphagnums are common about lakes, but absent or very rare along the stream. In both cases, however, there is a dominance of northern forms.

2. FLOODPLAIN SOCIETIES.—The series of changes taking place in the previous group are continued until swamps and pools disappear, to be replaced by a dense woods of mixed mesophytic species. Three societies appear, often with a more or less zonal arrangement.

(1) *Moist sedge society.*—Turf-forming grasses here make their first appearance, but are not able to replace entirely the terrestrial sedges, which occupy occasional areas.

(2) *Elm-ash-maple society.*—There is little space not occupied by tree growth, except where clearings have been made. *Populus tremuloides* and *P. deltoides* appear early as pioneers, followed later by *Ulmus americana*, *U. pubescens*, *Fraxinus pennsylvanica*, *F. lanceolata*, *F. nigra*, *Acer saccharinum*, *A. rubrum*, *Platanus occidentalis*, and *Tilia americana;* which make up a woods characteristically mixed, dense, and vine-clad. Of the herbaceous forms, only the most shade-enduring kinds with broad thin leaves are to be met with in the forest. Of these *Urticastrum divaricatum*, *Acnida concatenata*, *Adicea pumila*, and *Lobelia cardinalis* are most common.

(3) *Walnut society.*—The most abundant and characteristic tree of this association is *Juglans nigra*, which appears to have been uniformly distributed, and often over three feet in diameter, as shown by stumps. It is one of the arboreal types which shows a tendency to follow along streams or bodies of water. It occurs mixed with *Juglans cinerea*, *Quercus macrocarpa*, *Hicoria minima*, *Quercus platanoides*, and other species. It was attempted to characterize the group as a bur oak-walnut association. This was

abandoned because the bur oak was found to occur not alone in moist situations, but with equal frequency under much more xerophytic conditions, such as prevail in the oak openings a few miles west from Ann Arbor. However, the bur oak of these drier situations differs in appearance from the walnut association form, which has drooping branches with heavy corky ridges. The walnut society occurs in the better drained parts of the floodplain and for a short distance up from the base of the slopes, as shown in the map. It grows best of all in the coves. Along the tension line nearest the water *Carpinus caroliniana* is common; along the drier tension line *Ostrya virginiana* is rarely absent. The most common associated herbaceous species are *Houstonia ciliolata*, *Mesadena atriplicifolia*, *Allium canadense*, *Verbesina alternifolia*, *Homalocenchrus virginicus*, *Falcata comosa*, and *F. Pitcheri*.

As a whole the vegetation of the floodplain is mesophytic. Its growth differs from that of the peat-forming group in being mainly forest, in the tendency of its species to grow in mixture, in possessing a greater number of southern forms in its composition, and in the greater shade endurance of its species. The composition does not vary much in similar parts of the valley, but certain elements occur which serve to distinguish the stream-course type from that which occurs in the more remote parts of the valley, where mesophytic associations are found. *Platanus occidentalis*, *Juglans nigra*, *Hicoria laciniosa*, *Houstonia ciliolata*, *Quercus acuminata*, *Meibomia canadensis*, *Fraxinus pennsylvanica*, *F. lanceolata*, and *Acer saccharinum* occur characteristically along stream; but *Fagus americana*, *Acer Saccharum*, *Asimina triloba*, and associated species, forming a distinct maple-beech society in some other parts of the valley, are rarely met with on the floodplain.

3. BLUFF SOCIETIES.—Like the floodplain, the bluffs are covered with forest, but of a quite different character. The woods are more open and share in common with other vegetation numerous xerophytic adaptations. Four societies need to be distinguished in an account of existing relations.

(1) *Oak-hickory society.*—Beyond the few hickories at the north, this society is little represented at the bayou. This may be partly explained by the absence of the more moist till slopes, upon which

...COLORADO... STATE ... SCHOOL

it is abundantly represented in the vicinity. A variety of hickories is found, most common of which are *Hicoria ovata*, *H. alba*, *H. odorata*, and *H. glabra*. These are associated with *Quercus rubra*, and *Q. alba* is present.

The other vegetation also shows a number of easily recognized characteristics. The proportion of leguminous species (Melilotus, Trifolium, Vicia) is high; composites (*Aster laevis* and other summer-blooming species) are frequent; *Podophyllum peltatum*, Erythronium, *Muhlenbergia diffusa*, *Scrophularia marylandica*, and *Cornus candidissima* are common species. Counting in the smaller vegetation, the society is not rich in species, but is rich in southern forms.

(2) *Black oak society.*—The cemetery bluff conditions, brought about partly by the porous sand and partly by slope, are much more arid than the till bluffs. The hickories and red oak disappear from the forest, and *Quercus velutina* becomes most common, 53 per cent. of the individuals on the slope above the 760-foot contour line being black oaks. *Quercus alba* is common, and in places west of Ann Arbor *Q. imbricaria*.

Comparing the other vegetation with that of the oak-hickory society, it is found that equal differences exist. The May apple is rarely found; there are fewer leguminous species; ericaceous shrubs are common; and the proportion of composites is high. The vegetation is rich in species, characteristic of which are *Lespedeza capitata*, *L. violacea*, *Solidago caesia*, *S. speciosa*, *S. nemoralis*, *S. rigida*, *Gaultheria procumbens*, *Gaylussacia resinosa*, *Vaccinium vacillans*, *Angelica villosa*, *Viola pedata*, *Lupinus perennis*.

All of the numerous species of grass which clothe the steeper slopes have prominent adaptations serving to bind down and hold the soil from outwash. They are all perennials, have deep root systems, strong woody stems and rhizomes, and grow in compact tufts which offer the greatest resistance both to being torn apart and to being displaced. Even loose sand, which otherwise would be quickly washed away, is by this means held in place indefinitely, and the steepness of slope so formed is often surprisingly great. It was found that the angle of repose of the dry sand composing the bluff was 28° 35′. The mean angle of the slope was 39° 55′; or the actual slope of the bluff as held by the soil binders is 11° 20′ steeper than

the dry, loose sand would take by gravity alone. *Andropogon scoparius*, *A. furcatus*, *Chrysopogon avenaceus*, and *Stipa spartea* are the most common of these soil binders.

(3) *Juniper heath.*—The conifers are restricted to open or unfavorable situations, and thrive where other arborescent forms do not. Only isolated individuals of juniper and cedar are met with at the bayou; but southeast from the city are found slopes covered by them. On the brink of a bluff which has been undermined by the stream so as to leave the slopes bare of any vegetation, *Juniperus communis* makes an early appearance. From this point it throws out decumbent rooting stems, which, partly directed by the movement of the unstable soil, come to extend down the slope, and the growth is continued from above downward. A covering is soon made, in which also occur *J. virginiana*, *Lepargyraea canadensis*, *Rhus aromatica*, and *Stipa spartea*.

None of the other societies occur under conditions equally xerophytic. The juniper and cedar are most common (*fig. 4*), but not so characteristic of such situations as other less frequent members, like buffalo berry or porcupine grass. If we compare slopes of different ages, evidence will be found showing that the juniper slope is transformed into an oak slope as conditions become more favorable for the growth of vegetation. In the heath appear *Populus grandidentata* and *P. tremuloides* and seedlings of black locust, basswood, and oak. Cedar Bend, Ann Arbor, has gone a step farther. Here the oaks are dominant, but there is still much juniper, cedar, and aspen. Even the final black oak stage is not without decayed logs, stumps, and isolated individuals, suggesting the former prevalence of the juniper and other members of that society.

(4) *Thicket societies.*—One of the most characteristic features of the river vegetation as a whole is the large proportion and variety of shrubs which it contains. Along the bluffs these shrubs occur either as scattered individuals or mixed together as thickets or undergrowth. The numerous species of thorn and bramble, Ampelopsis, Smilax, Celastrus, Vitis, and other vines, occur most frequently in dense mixed thickets, where an open ledge or slope affords a chance. *Rhus hirta* and *R. glabra*, however, do not tend to mix with other shrubs, or even with each other, but form patches of pure growth, which distinguish at once the sumac thicket from all others.

As a whole, the bluff vegetation is made up of light-requiring species. The prevailing forest trees are among the least shade-enduring kinds to be found in the region. Such trees never crowd together to form the dense type of woods common on the floodplain; but, like the black oak woods, or like the openings of bur oak west of Ann Arbor, the trees stand far enough apart to permit an abundant

FIG. 4.—Washed slope in juniper-heath stage; the ground juniper appears in patches in the foreground, with red cedar forming a dense grove in the background; near Rawsonville, Michigan.

growth of smaller vegetation to spring up in the woods. Also there is less tendency to growth in mixture. The sumac and junipers are gregarious, and there is not the variety of trees in the oak woods that there is in case of the more mesophytic kinds. The tendency of the black oak group to occur on sands, and of the hickories to occur on clays, are among the most constant relations which societies have been found to bear to soil types.

OTHER BIOLOGICAL GROUPS.

The account thus far deals mainly with indigenous plants which seem to bear a more or less constant relation to the environment, and includes a total of 316 species which have been classified into societies. There remain eighty-four species found upon the area surveyed, which occur in widely varying situations. These have been classified into two groups.

1. THE HETEROPHYTES.—A group of twenty-one native species were found to occur in so wide a range of habitat as to be best termed heterophytes. The most common of these are *Onagra biennis*, *Taraxacum Taraxacum*, *Poa pratensis*, *Aster novae-angliae*, and *Solidago canadensis*.

2. INTRODUCED WEEDS.—A second group, the exotics, embracing the introduced weeds, is characterized by a similar wide range of habitat. Their presence is due solely to the agency of man, and serves to indicate the extent to which he has changed the composition of the original vegetation by clearing away the forest and cultivating areas in the vicinity. Nearly one-seventh of all the species collected at the bayou have been introduced. Of these fifty-two are European, four (*Amaranthus retroflexus*, *Chenopodium ambrosioides*, *Mollugo verticillata*, *Datura Tatula*) are tropical American; one (*Ailanthus glandulosa*) Chinese; one (*Lonicera Tatarica*) northern Asiatic; one (*Abutilon Abutilon*) southern Asiatic. The important fact is brought out in the study of the bayou vegetation that the aquatic and swamp societies are almost free from introduced species. Only five species were found in this area: *Solanum Dulcamara*, *Mentha piperita*, *Roripa Nasturtium*, *Salix purpurea*, and *S. alba vitellina*. These were confined mainly to the drained swamp. The juniper-heath is also nearly free from exotic species. The largest number of exotic species occurs in the grain-bearing or grass-producing societies, the highest percentage being associated with the black oak and the oak-hickory societies.

PROMINENT INTER-RELATIONS.

From the fact that members of a plant society live together under common conditions, it is to be expected that individual species of a group would possess common adaptations and hence resemble one

another. This is seen to be the case in the tamarack society, for example, where there is a marked morphological resemblance. But it is possible to trace the resemblance much further. With this in view, the percentages of spring, summer, and autumn blooming species were obtained for each society. Fall percentages were taken as positive, summer percentages as zero, and spring percentages as negative, the results being shown in the accompanying table. A

TABLE SHOWING PERCENTAGE OF SPRING, SUMMER, AND FALL BLOOMING SPECIES; AND THE PERCENTAGE OF NORTHERN, CONTINENTAL AND TEMPERATE, AND SOUTHERN SPECIES IN EACH SOCIETY.

Species			Time of blooming							Geographical distribution						
No.	Society	No. of sp.	Spring −		Summer o		Fall +		Value	Northern −		Temperate and Continental		Southern +		Value
			No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
1	Juniper-heath..	5	5	100	0	0	0	0	−100	4	80	0	0	1	20	−60
2	Black oak.....	62	15	24	45	73	2	3	−22	4	6	29	47	29	47	+41
3	Sumac.....	2	0	0	2	100	0	0	0	0	0	2	100	0	0	0
4	Oak-hickory...	26	8	31	17	65	1	4	−27	0	0	15	58	11	42	+42
5	Walnut.....	50	20	40	30	60	0	0	−40	2	4	25	50	23	46	+42
6	Elm-ash-maple	46	22	48	24	52	0	0	−48	6	13	31	67	9	20	+7
7	Moist sedge....	3	1	33	2	67	0	0	−33	1	33	2	67	0	0	−33
8	Tamarack.....	13	2	15	11	85	0	0	−15	9	70	4	30	0	0	−70
9	Willow.....	71	32	45	39	55	0	0	−45	28	40	32	45	11	15	−25
10	Water sedge...	19	3	16	16	84	0	0	−16	5	27	13	68	1	5	−22
11	Typha-Sparganium.	10	3	30	7	70	0	0	−30	0	0	8	80	2	20	+20
12	Water smartweed..	6	0	0	6	100	0	0	0	0	0	4	66	2	34	+34
13	Waterlily.....	5	0	0	5	100	0	0	0	0	0	5	100	0	0	0
14	Pondweed.....	6	1	17	5	83	0	0	−17	0	0	6	100	0	0	0

curve was then plotted from the summation of such percentages. The curve suggests that in general similar societies possess similar habit. The societies which bloom earliest have a high per cent. of northern forms. Like many of the willows, poplars, and maples, the flowers often appear before the leaves. Also the leaves unfold and the fruit matures before more southern types like the oak, hickory, walnut, and sycamore. The bluff societies, except for the juniper, possess the southern habit.

The trees are mainly nut-bearing and ripen their fruits, unfold their leaves, and bloom relatively late. Thus we commonly see a tamarack swamp or a floodplain forest with young foliage well along before the surrounding oaks have begun to show marked signs of life. Both curves show that the highest per cent. of vernal as well as northern forms occurs in the floodplain societies and on the juniper slopes. The highest per cent. of southern forms occurs along the favorable slopes of the bluff and in the aquatic societies.

CONTROLLING FACTORS.

1. CLIMATIC.—A number of species have been pointed out whose distribution is more or less restricted to the stream, although this will be shown to be less true near the mouth. Most common of these are the hickories, walnut, yellow oak (*Q. acuminata*), red elm, silver maple, red and green oak, sycamore, hoptree, *Rhus aromatica*, *Juniperus communis*, *J. virginiana*, *Iris versicolor*, *Meibomia canadensis*, *M. grandiflora*, *Ambrosia trifida*, *Dioscorea villosa*, *Sanguisorba canadensis*, and *Mesadenia atriplicifolia*. Most of these are near the northern limits of their range. Since many of these forms occur in a wider range of habitat in the south, it may be assumed that climate plays some part independent of other factors in causing such species to become limited to the neighborhood of the stream.

2. ATMOSPHERIC.—The fact that the forest is usually open along the edge of the bluffs seems to account in large part for the presence here of the juniper and associated species. Their behavior indicates exacting light requirements, as they thrive only in open situations, and early show a decline when shaded. To a less degree the sumac society occupies open places in a similar manner.

3. MECHANICAL.—The mechanical effects of stream erosion are continually bringing about changes of topography and soil to which the plant societies are in turn conforming. Although a number of bluffs are being denuded by the cutting of the current, the floodplain areas are not likely to be very greatly increased; since it has been shown that a stream cuts within the limits of a belt not over eighteen times the width of the stream at that point.[3] The fact that Populus

[3] JEFFERSON, M. S. W., Limiting width of meander belts. Nat. Geog. Mag. —:373–384. 1902.

and Salix are often the first woody types to appear on the exposed places of the floodplain and bluffs appears to be due to nothing beyond the fact that they were first on the ground. Judging from the shrubs, dispersal by wind may be more rapid than dispersal by animals, but it is certainly far less prevalent, as will be shown later.

4. EDAPHIC.—Of all the local conditions, the societies sustain the most obvious relation to the amount of available moisture in the soil. It is this which in large measure gives the distinctive characters to the bluff societies as compared with those of the floodplain or the peat-forming group. The relation which tension lines bear to water supply takes on particular interest in case of the bayou peat-forming societies indicated in the map. There is a slope toward the center in which there occurs a pond-like bay. The main part of the surface water discharged from the seepage springs along the cemetery bluff is carried to the southeast to a point near the fence, where it makes a good sized stream as it flows directly to the ponds. A similar discharge of surface water comes from several points along the elm-ash-maple zone. The map shows the general tendency of tension lines to arrange themselves concentrically about the bay; also a tendency to arrange themselves parallel to the stream, and the complex pattern of the peat-forming societies is the resultant of these conflicting tendencies.

A chemical analysis was made of the soil water about the springs in the hope that peculiarities might be detected to account for the xerophytic adaptations noticed in the vegetation. The water was found to be neither acid nor alkaline. It contains sulfates, chlorids, and carbonates, with iron, calcium, and sodium, but not different from the usual spring water. The water, however, is cold and keeps the swamp at a low temperature. There are five of the societies which stand in so definite a relation to soil types that they give fairly reliable information as to the kind of soil merely by their occurrence. The black oak society may be taken to indicate dry sand or gravels; the oak-hickory society, till or clay; the tamarack society, peat; the elm-ash-maple society, wet soils; the maple-beech society, richness of soil. Such soil relations do not appear to be limited to the valley alone, but to hold true over a considerable area outside.

5. BIOTIC FACTORS.—It is due to the interaction of one life form

upon another that there have been produced areas of turf which otherwise would have been very limited or wholly absent. Along the brink of the cemetery bluff a line of tension is easily traced. It is not sharp like the swamp lines; but, like the usual upland type, there is an area over which the societies blend. *Poa compressa*, *Andropogon scoparius*, *A. furcatus*, and *Stipa spartea* crowd their way for a distance of one to six feet into the turf of *Poa pratensis* and *Trifolium repens*, where they fade out altogether.

It is plain that animal life has played an important if not a chief part in the dispersal of seeds in the valley. Squirrels and like animals were formerly more common than now, but birds are yet numerous; the blue-jays,[3] cat bird,[4] and crow blackbird[5] alone feed upon a variety of fruits that includes more than one-half of the woody species at the bayou, exclusive of the large trees.

Besides these, there are the red-winged blackbird, vireo, oriole, woodpeckers, song sparrows, cowbirds, and robins, which are common all along the stream and are known to devour fruits. This has resulted in scattering the seeds of a number of shrubs, vines, and small trees in the open places of the forest. At the bayou there are sixty-one species of this kind, 82 per cent. of which have adaptations for seed dispersal by animals; 72 per cent. have the brightly colored fruits eaten by birds, of these 22 are red, 10 black, 7 blue, and 5 white. It is possible that this may indicate something with regard to the relative attractiveness of the several colors.

Over two-thirds of the species with colored fruits occur in the bluff societies, and are most numerous with the juniper, sumac, and black oak. This fact is not so easily explained by any habit of the birds as it is by the character of the woods. The same relations appear to hold true of corresponding societies away from streams,

3 BEAL, F. E. L., The blue-jay and its food. Yearbook, U. S. Dept. Agric. **1896**: 202.

4 JUDD, S. D., Birds of a Maryland farm. Bull. 17, U. S. Dept. Agric. Div. Biol. Survey, p. 104.

5 BEAL, F. E. L., Food of the bobolink, blackbirds, and grackles. Bull. 13, U. S. Dept. Agric., Div. Biol. Survey, p. 64.

——, The crow blackbirds and their food. Yearbook, U. S. Dept. Agric. **1894**: 243.

where the forest has been undisturbed. Blueberry, barberry, blackberry, hackberry, huckleberry, raspberry, strawberry, currants, cornel, cedar, juniper, sumac, sassafras, black cherry, pin cherry, choke cherry, and black haw occur commonly in the dry sand of oak woods, less commonly with the hickories, and least of all in the maple-beech woods. Oaks, however, have thin crowns, are not shade-enduring, and do not grow crowded together, forming open woods that give a chance for the growth of a variety of herbs, shrubs, and vines on the forest floor. On the other hand, the beech, maple, and ash are distinctly shade-enduring, have thick crowns, and grow crowded together, forming a dense woods in which there is little chance for undergrowth to come in. In such woods there are so few shrubs and vines that the average woodsman is not aware of their presence at all, and finds himself at a loss to account for the thick berry patch that springs up wherever he cuts a few trees. It is plain that seeds are scattered in all woods, but only the open woods afford a chance for their growth.

6. THE HISTORIC FACTOR.—The presence of Sarracenia near cold seepage springs, and of the white pine on the bluffs, suggests that those plants may be relics of a past northern flora, which followed closely the retreat of the ice. While the tamarack society probably does not date back to this period in its origin, its flora may have been derived in large part from species so left, in the same way that the juniper-heath spreads out over exposures as fast as made on the bluffs. The fact that 70 per cent. of the species associated are northern adds confirmation, and accounts for the dissimilarity between it and neighboring societies. With further investigation it does not seem improbable that many if not all of the peat bog forms may be found lurking in the neighborhood of springs of this character.

Societies of the favorable places of the floodplain and bluffs have a pronounced southern flora. A few northern forms occur in all the floodplain societies; but the oak-hickory society, in which there occurs a high per cent. of southern forms, has so few northern species that their occurrence may be regarded as accidental. The curve (*fig. 5*) shows how in land situations southern tendencies fade out as conditions become more and more unfavorable.

SUMMARY

1. The bayou of Ypsilanti, as indicated in the sketch of its geological history, is of relatively late origin, and relics of postglacial floras have been nearly obliterated by more recent changes, which render the portion of the valley directly affected by the stream not so suitable for the preservation of ancient floras as the secluded swamps

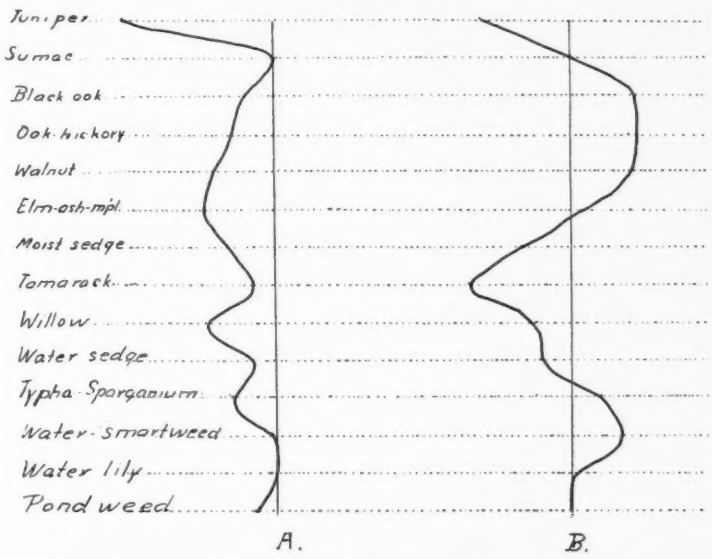


FIG. 5.—*A*, curve of period of bloom; *B*, curve of geographical distribution; curves drawn from values obtained in map.

or bogs of the morainal regions. But the seepage springs and bluff exposures of sufficient age and isolation still retain such relicts of a past northern flora which followed closely the retreat of the ice.

2. The societies of river swamps, such as the bayou here described, are distinct from those of the lake swamp or bog. In both, however, xerophytic adaptations are conspicuous, which cannot be explained by the absence of drainage currents nor by the presence of humus acids.

3. The peat-forming societies show sharp tension lines, conforming to depth of water, characteristic of pond vegetation. Such tension

lines become obscured in the floodplain societies, and still more so in the bluff societies, but in each of them the relation of distribution to soil water, as a controlling factor, is plainly marked. The definite relation of certain societies to soil types, shown to exist here, appears to be due primarily to the capacity of these various soils for water.

4. Of other factors to which the plant societies are evidently related, the influence of light is conspicuously manifest, as for example in the place taken by light-requiring species in the bluff vegetation. Quite as manifest, though far more complicated, is the coincident operation of biotic factors, which are so numerous and varied in their manifold interrelations as still to demand much special study.

5. The high per cent. of northern species in early blooming societies, the occurrence of various southern forms along the river near the northern limit of their range, the occupation of favorable places by societies of distinctively southern cast, and of unfavorable ones by those of pronounced northern composition, are all indicative of the close relation of the members of these societies to slowly changing climatic conditions. A discussion of the migrations of these plants in connection with geographic and climatic changes is deferred until a greater accumulation of data has been made.

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THE SPORE COATS OF SELAGINELLA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXX.

FLORENCE LYON.

(WITH PLATES X AND XI)

SINCE the development of the modern technique in botany, comparatively little has been done in investigating the mode of formation of cell walls or of spore coats, and botanists are still much in the dark as to the nature, as well as the morphology, of certain complex structures protecting the protoplasm of spores. A recent contribution to this subject by FITTING[1] is of special importance, because as a result of his study of the development of the spore coats of certain species of Selaginella, he advanced a theory that they grow quite independently of the protoplasm of the spore, with which during the time of their greatest increase in bulk they have no organic connection except at one point. This, of course, is revolutionary and quite at variance with all previous ideas of the growth of membranes; and if it is true that protoplasm "can act at a distance," and without organic connection can build massive structures, the fact is of the greatest importance.

At the time that FITTING'S paper appeared, I was investigating certain phases in the life history of two native species of Selaginella—*S. apus* and *S. rupestris*. In the main the peculiar phenomena connected with the formation of the spore coats of the first named species seemed to agree with those described by FITTING; but the development of the spores of *S. rupestris* differed so completely that it became a matter of interest to determine whether there were two distinct types of spore formation in this genus or whether the differences were more apparent than real. The present work was undertaken with a view to throwing some light upon this question. The difficulties which all investigators have encountered at every stage

[1] FITTING, HANS, Bau und Entwickelungsgeschichte der Makrosporen von Isoetes und Selaginella und ihre Bedeutung für die Kenntniss des Wachsthums pflanzlicher Zellmembranen. Bot. Zeitung **58**[1]: 107–164. 1900.

of technique, fixing, embedding, sectioning, and staining, together with the fact that Selaginella spores are *sui generis*, and one has no standard of comparison, make one fearful of drawing conclusions from artifacts. Apparently the only way to lessen this danger and make results convincing, is to have a very large number of preparations killed and treated in various ways, for little critical study can be made of the living spore. This has made my investigation an exceedingly tedious one, for in the early development of the spore, transformation takes place so rapidly that important phases are obtained with difficulty, and if not found the danger of misinterpretation is very great. The results of my work fail to confirm FITTING'S theory, nor am I able to accept his description of the origin of the coats for the species that I have investigated. It is quite certain, I think, that the spore coats of *S. rupestris* do not lose their complete organic connection with the protoplast during growth, and I have a growing conviction that to our imperfect technique are due the phenomena which gave rise to FITTING'S theory and were described by FITTING, CAMPBELL, and all other workers in this group, including myself.

Very complete series of three species—*S. apus*, *S. Emmeliana*, and *S. rupestris*—from the origin of the sporangium to the development of the embryo sporophyte have been studied. Throughout its history *S. rupestris* is aberrant, and proves to be a plant of much importance and interest, as it has characteristics supposed to be peculiar to the seed plants. Normally, and in a greater number of cases, only two megaspores were found to develop in a sporangium. These may be two of a tetrad, the others failing to develop after the division of the mother cell; or, more important still, the mother cell may divide only into two spores. These megaspores are not shed from the megasporangium at all! The female gametophytes and the young sporophytes are retained in the old strobilus until the latter have roots, stems, and leaves; then the tissue of the sporangium and megasporophyll decays, thus liberating the bodies that lack only integuments to be seeds. A still further reduction of megaspores to *one* is infrequently found. In tracing the growth of the spores, I again emphasize the fact that at no time is there any appearance which would suggest that the protoplasm is not in con-

tact *at every point* with the thick gelatinous membrane from which the coats are formed.

The megaspore mother cell in all species may be distinguished from the other sporogenous cells (*fig. 1*) at the time that the sporangium wall is differentiated into three layers. It is slightly larger, more granular, and stains intensely as contrasted with its sister cells. Its wall thickens slightly, stretching and probably growing somewhat, and persists until the enclosed spores are more than half grown. I shall refer to it as the *mother cell membrane*, to distinguish it from a second membrane, the *spore membrane*, that shortly appears upon its inner surface in contact with the protoplasm (*fig. 1*). The mother cell membrane remains comparatively thin; whereas the spore membrane is gelatinous in nature, becomes very massive, and dissolves readily in many of the fixing reagents, especially in very young stages. It increases rapidly in bulk, forming a thick layer about the protoplast which for a period grows imperceptibly, if at all (*fig. 2*). As the mother cell divides, it assumes a dumb-bell shape, the two resulting spores often not completely separating from each other until half grown (*fig. 3*). At this early stage, each spore consists of a protoplast with a relatively large nucleus, and an envelop of thick gelatinous matter, the spore membrane. The two spores lie within the mother cell membrane. Whether any part of the spore membrane is formed in connection with a nuclear plate at the time of cell division could not be determined, owing to the diminutive size of the spindles and their infrequency in the material investigated. The protoplast becomes vacuolate. Many small vacuoles ultimately coalesce to form a very large one centrally placed, so that the protoplast comes to be a delicate vesicle (*fig. 4*).

The first indication of a coat is detected as a transformation of a portion of the gelatinous spore membrane. A clear homogeneous region at some little distance from the protoplast stands out in contrast to the rest of the membrane, which has become granular (*fig. 4*). This area soon becomes convoluted upon its outer surface, and these convolutions coincide with radiations in the outer granular portion of the membrane that suggest fixed diffusion currents (*fig. 4*) of solutions passing through the two membranes from the sporangium. At this time, the sporangium is turgid with fluid made up of dis-

organizing sporogenous cells, and a secretion poured out by the tapetum, which is the source of nourishment of the two young spores.

The spore membrane itself is a somewhat viscid substance, almost fluid in character, that flows out of the hole if a living sporangium be pricked with a needle. The spore membrane becomes much thicker at the base of the spore, so that the remarkably small protoplast lies near the apex (*fig. 5*). The growth and differentiation of the spore membrane into regions are extraordinarily rapid. Just inside of the clear area described above, a part of the membrane is much denser than elsewhere. These two regions, *i. e.*, the clear and the denser (*fig. 5*), constitute the preliminary stage of the first or outside coat, the *exospore*. It is possible in a close series of preparations to note gradual changes in structure as the coat matures. Chemical tests to determine the nature of the transforming spore membrane and of the resultant coats were very unsatisfactory. In all early stages the minute living spores are so difficult to manipulate that no reliable results could be obtained. On the other hand, material that is sectioned and upon the slide where it can be handled has already been subjected to the action of many reagents. The clear area, or outer layer of the exospore (*fig. 6*), is gradually transformed into a granulated condition, while the inner layer and the rest of the spore membrane becomes reticulated. All the regions are quite distinct and react differently to the stains used.

Up to this time the protoplast has not altered in size or density, but now it suddenly dilates—like a bubble which is blown up—and presses outward toward the exospore. When its diameter is about half that of the entire spore, a second delicate coat, the *endospore*, may be detected, having formed upon the outer surface of the protoplast. This is difficult to demonstrate unless the coat and protoplast become torn apart and displaced; for it is little more than a faint line in section during the entire period that the protoplast is expanding and pressing it back against the outer coat. The portion of the spore membrane (*fig. 6*) which is not directly transformed into the exospore, and against which the protoplast presses (*fig. 7*), disappears as the protoplast and endospore dilate. As the exospore has been undergoing modifications (*figs. 7* and *8*, *ee*) during this

time, which render it more impervious to the entrance of solutions from the sporangium, this undifferentiated portion of the membrane in its turn has probably served for nutriment or has been taken into the protoplast. Often, however, a little of the original membrane may be detected crowded back against the exospore, forming a layer that easily may be misinterpreted as an additional coat, if intervening stages have been overlooked (*fig. 8, h*).

As the spores now nearly fill the sporangium cavity and the tapetal cells are disorganized, all future growth must be at the expense of the central vacuole. The principal changes in the maturing of the spore are the increase in the amount of protoplasm, with marked growth of the nucleus, and the thickening of the endospore by successive laminae upon its inner surface, which is in contact with the protoplast at first only in the region of the nucleus (*fig. 9*). Eventually, however, it becomes a layer of equal thickness throughout (*fig. 10*). The exospore is elaborately bossed and sculptured, and the protuberances form a layer—the so-called perinium—that stains quite differently from the part in contact with the endospore (*figs. 7-10*). It seems to me from its formation that it should not be regarded as a distinct coat, however, but as the outer region of the exospore. The central vacuole throughout the spore development is filled with a transparent fluid, which in early stages disappears in preparing the slide, but which becomes a coagulable mass as the female gametophyte forms (*fig. 11*).

The most conspicuous differences between *S. Emmeliana* or *S. apus* (taken as a type) and *S. rupestris*, described above, are due I think to the *simultaneous* transformation of the spore membrane of the former two coats, and to certain phenomena due to mechanical strains that arise between these two coats and the protoplast. In *S. Emmeliana*, as in all other species hitherto studied, the mother cell normally divides into a tetrad. The same difficulty arose as in the preceding species about determining the origin of that portion of the spore membrane on the contiguous surfaces (*fig. 14*). Earlier stages show the formation of nuclear plates, and there is no such appearance of lobing of the spore mother cell as in *S. rupestris*. At first the spore membrane is homogeneous and translucent (*fig. 15*), but when its thickness is about half the diameter of the protoplast

(*fig. 16*), a portion of it lying in contact with the protoplast stains differently and marks the beginning of the two coats. This layer, almost as soon as it can be detected at all, shows a dual nature. It appears in section as a shaded band not sharply marked off from the undifferentiated portion of the spore membrane. Its outer region stains more vividly than the inner, perhaps because it is denser. Gradually a difference in structure becomes apparent, which I have attempted to show in *fig. 17*.

The spores are so small, stain so intensely during this critical stage, and the changes in the metamorphosing spore membrane occur with such rapidity that it has been with difficulty that I could demonstrate to my own satisfaction that one coat was not formed after the other, but both simultaneously. FITTING thinks that the endospore is formed after the first and arises *de novo* as a new formation of the protoplast. The spore is much smaller, and the changes much more rapid in this form; consequently they are harder to follow than in *S. rupestris*. The outer denser region with its roughened surface becomes the exospore, the inner the endospore. The protoplast becomes a vesicle by the formation of one large central vacuole. It is at this time when there are two coats of distinctly different structure that the peculiar phenomena occur which led FITTING to draw the conclusion that the protoplast is not concerned in the conspicuous growth of the spore coats.

As the spore grows larger, the outer coat increases in diameter and thickness much more rapidly than the inner, producing a strain in the viscid mass of which the two are concentric layers. FITTING thinks this results normally in a complete separation of the two, thus developing between them a comparatively large empty space. In a like fashion, due to the fact that the protoplast is quiescent and the endospore growing, a second "empty space" is formed between them, except at one point, thus severing all organic connection of the protoplast with the developing coats except at this point of tangency. There is no doubt that the majority of preparations have precisely this appearance. Moreover, the living spore examined in a normal salt solution seems to confirm FITTING'S view of a separation of the coats, and on this fact he lays great stress. The spaces, according to him, are afterward partially filled with a solution which has entered the spore

from the sporangium.[2] I have come to believe, however, that where actual gaps occur they are due to shrinkage and the dissolution wholly or in part of the intervening substance, and that the appearance of separation in the living spore can be explained by the different density of the concentric layers. In material showing no shrinkage elsewhere, I found that these regions were not empty, but completely filled with matter closely resembling the spore membrane at different periods of its transformation into coats.

It must be understood that the entire substance of the spore outside of the protoplast is a viscous mass of the consistency of thin syrup, in which two regions are becoming not only different in their physical but also in their chemical natures. This substance retains a plastic condition until the spore has attained its full size and the two coats their entire bulk. Then follow the changes which result in their hard horny or woody character. Into the viscid mass are passing solutions from the sporangium. The direct physical effect upon this mass as a whole would be a tendency to increase in diameter and bulk; but apparently all regions do not swell alike, the more resistant regions where coats are forming become drawn apart, having between them a stretched area of less dense nature, very easily dissolved by reagents, subject to great shrinkage and liable to be torn and not readily stained. From material fixed in bichromate of potassium and exposed to light, the gelatinous nature of the substance in these regions was clearly demonstrated. They always stain like the undifferentiated part of the spore membrane. FITTING also remarks that "sie verhalten sich bei allen Arten gegenüber Rengentien wie die Special Mutterzellmembranen."

There seem to be three possibilities: (1) FITTING's view that the regions between protoplast and under coat, and between inner and

[2] "Die Zwischenräume zwischen den Häuten und zwischen dem Mesospor und dem Plasma sind an lebenden Sporen ganz hyalin. . . . An fixirtem und mit dem Mikrotom geschnittenem Material sind in ihnen Gerinnungsmassen, je nach dem Entwickelungsstadium in verschiedener Menge, vorhanden. Wenn die Abhebung der Membranen gerade begonnen hat, ist von ihnen noch nichts zu sehen, erst wenn die Hohlräume etwas grösser geworden sind, werden in ihnen kleinere und grössere, hier und da netzartig verbundene sichtbar die während der Vergrösserung der Sporen schnell vermehrt werden. In noch älteren Stadien bilden sie . . . . sowohl zwischen Exospor und Mesospor, wie auch zwischen diesem und der Plasmablase je eine hyaline, glasartig durchsichtige Masse, die die beiden Räume meist nicht vollständig ausfüllt."

outer coat, are spaces filled with a solution—comparable to a vacuole; (2) that possibly there are protoplasmic connections between the protoplast and the two coats, and that the intervening substance is protoplasm, penetrating the endospore and extending to the exospore; (3) the suggestion, made above, that normally there are no clefts in the spore, but that the matter outside of the protoplast is to be regarded as an envelop of gelatinous matter in process of local transformation into concentric layers of different physical nature, and at the same time increasing enormously in size by the imbibition of matter from the sporangium. Much time was expended in trying to demonstrate protoplasmic continuity through the coats but without success.

The third view grows convincing, as it *is* possible to obtain sections which show the spores to be a solid mass without spaces, the regions of the developing coats not being sharply marked off from the substances that FITTING regards as foreign matter, and that I believe to be merely stretched areas of the gelatinous membrane out of which the spore coats are differentiating. Indirect evidence is afforded by the growth of the coats in *S. **rupestris*** in which there is at no time any suggestion of "spaces filled with nutrient solutions."

The further development of the megaspore consists in the expansion of the protoplast which soon overtakes the endospore, which in turn is carried along and stretched against the exospore. The portion of the exospore in contact with the endospore (*fig. 18*) usually fails to coalesce completely with the outer part (*figs. 18* and *19*), and if one has failed to follow its behavior closely might readily be interpreted as an intermediate distinct coat. This account fails to agree with FITTING'S and CAMPBELL'S.[3] The two coats I have shown in *figs. 17–19* they call exospore and mesospore. The endospore they describe as arising *de novo* after the protoplast is fully expanded, and in contact with it, thus making *three* distinct coats. In the species which I have investigated—*S. **rupestris**, S. **apus**, S. **Emmeliana**, S. **densa**, S. **cinerascens***, and two unnamed species from Jamaica—I can demonstrate only two distinct coats. May it not be possible that others have made the same error that I did in my

[3] CAMPBELL, D. H., Studies in the gametophyte of Selaginella. Ann. Botany **16**:419–4 8. *pl. 19.* 1902.

former paper[4] when I interpreted the inner region of the exospore as a separate membrane?

In a late paper by BEER,[5] the author states that he has observed a separation of coats in the pollen grains of certain Onagraceae that is quite comparable to the condition described by FITTING as occurring normally in the megaspores of certain species of Selaginella. His forthcoming paper on the subject will be one of interest, especially if he finds evidence to support FITTING'S view of protoplasm acting at a distance.

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EXPLANATION OF PLATES X AND XI.

In all figures, *a* indicates mother cell membrane; *b* the protoplast, *c* the spore membrane, *d* the nucleolus, *n* the nucleus, and *v* the vacuole.

PLATE X. Selaginella rupestris.

FIG. 1. Median section of megaspore mother cell with three sporogenous cells that fail to form spores. ×1250.

FIG. 2. Not quite median section of megaspore mother cell showing slight lobing, before division into two spores. ×1250.

FIG. 3. Nearly median section of two megaspores not completely separated from each other; that portion of the spore membrane between the two protoplasts is apparently formed by the lobing of the mother cell and not in connection with a nuclear plate. ×1250.

FIG. 4. Median section of megaspore after the formation of a large central vacuole and at the time the spore membrane shows a region differentiating into the exospore or outside coat; *e*, region differentiating into exospore; *f*, possibly diffusion currents of solution passing into spore from sporangium. ×600.

FIG. 5. Sections of two megaspores lying near the sporangium wall; by greater growth of the spore membrane at one side the protoplast has come to lie near the apex of the spore; the spore *A* is in nearly median section, *B* is not; *e*, exospore, *s*, sporogenous cells that failed to divide into megaspores. ×600.

FIG. 6. Portion of median section of megaspore (drawing not completed at base); to show further differentiation of spore membrane; *e*, exospore showing three different regions. ×600.

FIG. 7. Apical portion of median section of more advanced stage of megaspore to show the first appearance of the second coat—endospore—and the changes in the exospore; the undifferentiated part of the spore membrane (*h*)

[4] LYON, FLORENCE, A study of the sporangia and gametophytes of *Selaginella apus* and *Selaginella rupestris*. BOT. GAZETTE **31**:124–193. *pls. 5–9.* 1901.

[5] BEER, RUDOLPH, The present position of cell-wall research. *New Phytologist* **3**:159–164. 1904.

between the exospore and endospore shows signs of dissolution; *c*, spore membrane (includes everything between *a* and *g*); *e*, portion of spore membrane becoming transformed into exospore; *g*, endospore. ×600.

FIG. 8. Small portion of median section of apical region of megaspore more advanced; the protoplast *b* with the endospore *g* on its outer face has expanded until it has come to lie close to the outside *e;* the undifferentiated part *h* of the spore membrane has almost entirely disappeared; *e*, exospore; *g*, endospore (black line); *h*, layer of granular matter which is all that remains of region between *c* and *g* in *fig.* 7. ×60.

FIG. 9. Apical region of median section of older megaspore; the mother cell membrane has disappeared entirely; *b*, protoplast increasing rapidly; *e*, exospore; *g*, endospore (increasing in thickness by successive laminae formed on its inner surface in the vicinity of the nucleus). ×600.

FIG. 10. Portion of apical region of median section of mature megaspore in the region of the nucleus; the protoplast has increased in thickness at the expense of the central vacuole near which the nucleus lies; the protoplasm contains much proteid matter which stains deeply; *e*, exospore; *g*, endospore. ×600.

FIG. 11. Part of median section of female gametophyte after cell walls have begun to form; the proteid granules have increased in number and in size; *e*, exospore; *g*, endospore; *nn*, nuclei. ×600.

PLATE XI. Selaginella Emmeliana.

FIG. 12. Megaspore mother cell in optical median section, after the formation of the spore membrane. ×1250.

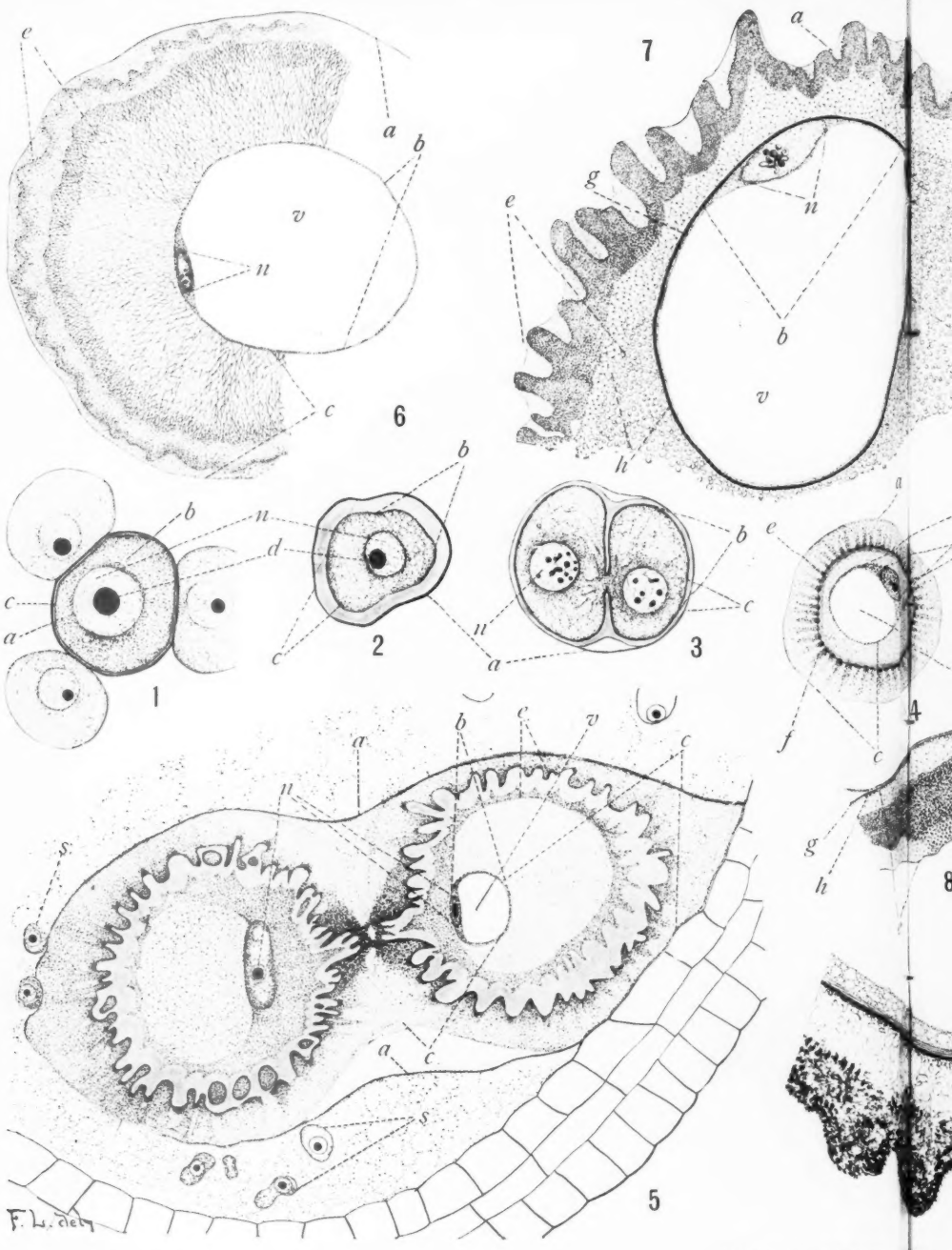
FIG. 13. Median section of megaspore mother cell in process of division into tetrad; nuclear plates forming; *x*, nuclear plate; *y*, spindle fibers. ×1250.

FIG. 14. Median section of megaspore tetrad showing completion of spore membrane along the nuclear plates. ×1250.

FIG. 15. Median section of somewhat older tetrad, showing increase in thickness of spore membrane between the protoplasts of the spores; protoplasm becoming vacuolate. ×1250.

FIG. 16. Median section of tetrad showing the differentiation of a layer of the spore membrane; the outer region of the shaded band with the convoluted border becomes the exospore, the inner region in contact with the protoplast becomes the endospore; outside of the forming exospore, between it and the mother cell membrane, is a layer of the undifferentiated spore membrane; *d*, portion of spore membrane in process of transformation into coats; *e*, region of exospore; *f*, region of endospore; *m*, undifferentiated portion of spore membrane. ×1250.

FIG. 17. Nearly median section of tetrad showing farther differentiation of exospore and endospore; two megaspores show the effect of the mechanical strain between exospore and endospore which has a tendency to split the exospore into two layers; stages between *fig.* 16 and *fig.* 17 were not found among several hundred preparations; the transformation of the spore membrane into coats is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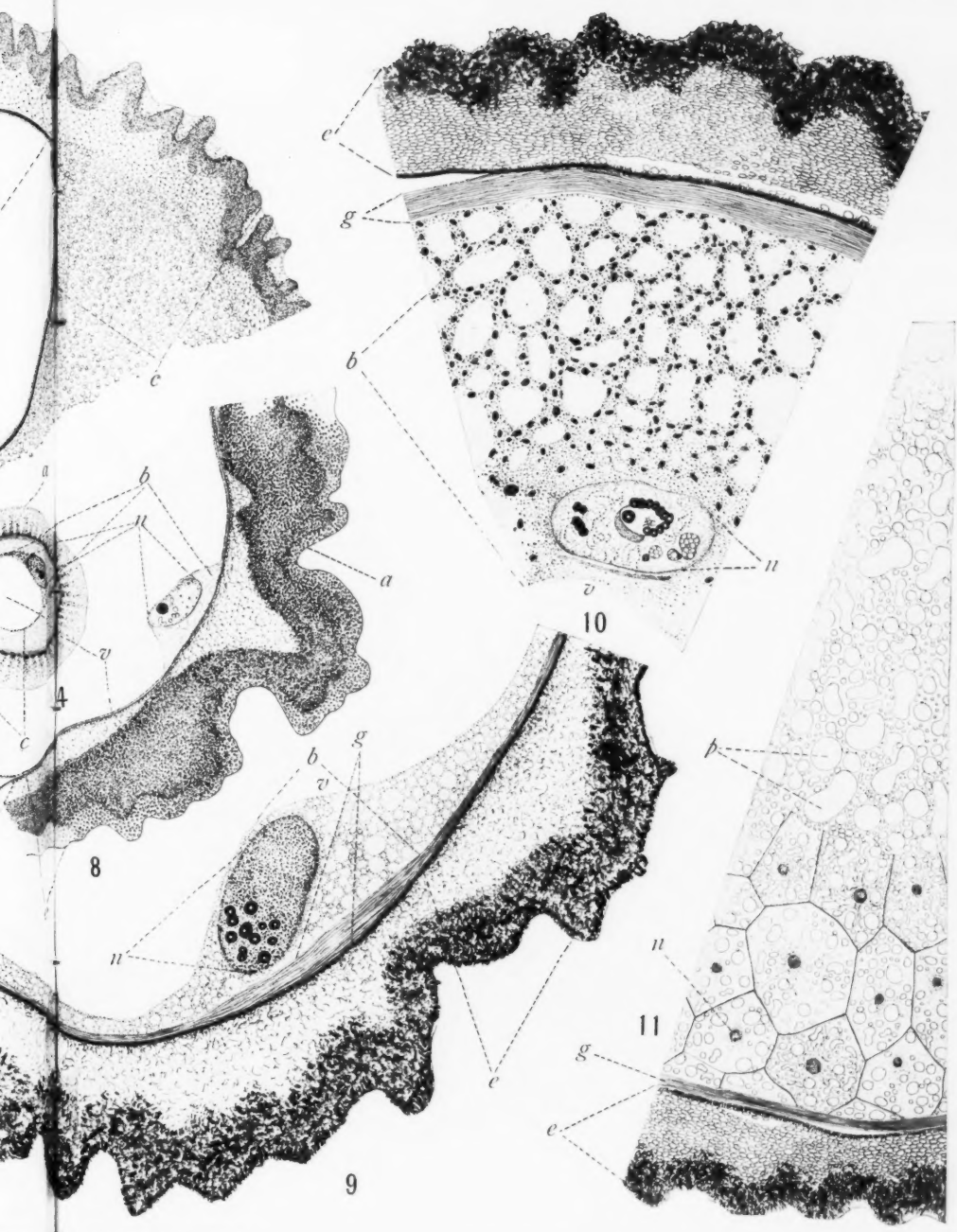

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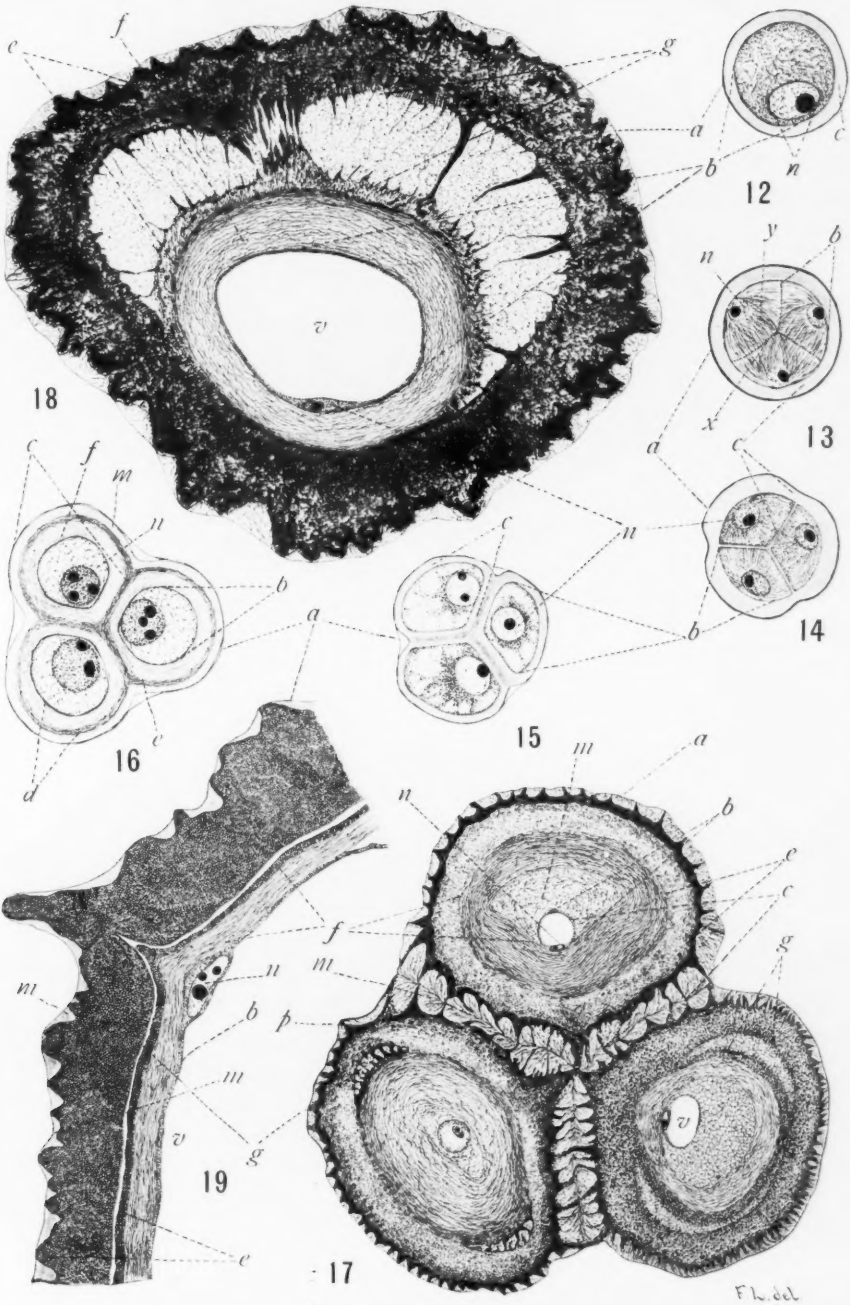

LYON on SELAGINELLA

very rapid; *e*, exospore; *f*, endospore; *m*, undifferentiated part of spore membrane; *p*, outer sculptured part of exospore (so-called perinium); *g*, strained region in exospore. ×375.

FIG. 18. Nearly median section of a megaspore at a later stage than *fig. 6;* the specimen was slightly shrunken, which gives the appearance of a cleft in the exospore traversed by some unbroken strands; the undifferentiated portion of the spore membrane between the exospore and the protoplast (*fig. 16*, *m*) has been transformed into endospore; at this stage the protoplast increases in diameter rapidly; *e*, exospore; *f*, endospore; *g*, stretched and partially split region in the substance of the exospore. ×375.

FIG. 19. Small portion of the median section of a nearly mature megaspore in the apical region; coats fully formed; the cleft separating the exospore into two layers is probably due to the action of reagents; *e*, exospore; *f*, endospore; *g*, cleft in substance of exospore; *m*, undifferentiated remnant of spore membrane. ×375.

CONTRIBUTIONS TO THE BIOLOGY OF RHIZOBIA. V. THE ISOLATION AND CULTIVATION OF RHIZOBIA IN ARTIFICIAL MEDIA.

ALBERT SCHNEIDER.

IT is with a somewhat guilty conscience that this paper is submitted. It is directly prompted by the fact that numerous inquiries have been received as to details regarding the isolation and cultivation of the root nodule microbe of leguminous plants. While I am now well satisfied that rhizobia are very easily isolated and grown in artificial culture media, I am nevertheless only too conscious of my earlier repeated failures and mistakes, and the failures of others. These facts should have prompted me to publish the finally successful efforts as soon as possible in order to assist and simplify the work of others who were contemplating research along similar lines. In a recent article by GROSVENOR,[1] outlining some rhizobia culture work done by Dr. GEORGE T. MOORE of the Department of Agriculture, the statement is made that "after much labor he (MOORE) isolated the nitrogen-fixing bacteria," from which we are led to conjecture that even at this late date similar difficulties were encountered. With perhaps the one exception of Miss DAWSON, so far as can be ascertained, no investigator has published a detailed description of the methods of procedure in the isolation and cultivation of rhizobia. It is then with the view of correcting this neglect that this paper is submitted.

The rhizobia of the various leguminous plants examined, isolated, and cultivated by the writer, such as those of red clover, white clover, bur clover, sweet clover, garden peas, and alfalfa, gave practically the same results so far as methods were concerned. The early difficulties and failures were due entirely to ignorance with regard to the behavior of these organisms in artificial media, as has been set forth in previous papers. However, in spite of the readiness with which rhizobia are cultivated, the student must observe certain pre-

[1] GROSVENOR, GILBERT H., Inoculating the ground. A remarkable discovery in scientific agriculture. *The Century Magazine* **68**:831–839 (October). 1904.

cautions in order that satisfactory results may be obtained. After numerous trials I have finally found the following methods to give the most satisfactory and most uniform results.

1. *Securing tubercle-bearing roots.*—Taking it for granted that it is desired to isolate and cultivate the rhizobium of some herbaceous leguminous plant, it is of considerable importance that the roots should be taken from plants growing in soil free from contamination with surface sewage, away from thickly populated areas, as vacant lots in large cities, etc. The object is to obtain tubercles which are comparatively free from foreign bacteria. Experiments with roots and nodules from plants growing in vacant lots in Chicago showed that they were covered externally with multitudes of microbes, which interfered very considerably with the rhizobia experiments.

Select healthy looking plants growing in loose soil, dig up the roots carefully; shake off the dirt very carefully, as the nodules are quite easily removed. By means of a sharp knife cut off root portions having well-developed single nodules or small groups (two or three) of nodules, and place them in a clean sterile container in which to carry them to the laboratory. Do not take roots with nodules that appear quite dark (bluish hue) at the base, as these are old tubercles and are quite badly contaminated with foreign soil microbes. Do not take roots with large tufts of nodules for similar reasons, and for the further reason that these nodule clusters are difficult to clean.

2. *Cleaning and garbling roots.*—At the laboratory look the material over carefully. Remove all foreign vegetable substances. Reject all roots or rootlets showing decay or discoloration. By means of the pocket knife cut away root portions which are undesirable. Now place this carefully garbled material under a faucet and let the water run over it to wash away dirt and sand. Care is necessary, as a strong flow or careless handling may remove the most desirable nodules. Remove all dirt, using a small brush, if necessary, to remove clinging sand and soil particles.

3. *Selecting nodules.*—After this cleansing, look the material over carefully. By means of a pair of small, blunt-pointed, clean, sterile tweezers pick off young, clean-looking, but well-developed nodules and drop them into a small, clean, sterile beaker about half full of thoroughly boiled water (boil this water yourself for half an hour).

About ten nodules should be selected. This is more than is required but allowance should be made for loss through accident.

4. *Brushing and washing nodules.*—Take up each tubercle by means of the blunt tweezers and clean it by means of a sterile camel's hair brush, rinse back and forth in the water, and then drop the cleansed nodules into a second small beaker half full of boiled water. Stir the tubercles about in this second beaker by means of a sterile glass rod or a small section lifter. By means of the section lifter transfer the nodules to a sterile test tube, about half full of boiled water. The camel's hair brush removes many microbes and sand and soil particles which cannot be removed in the following rinsing process.

5. *Rinsing the nodules in the test tube.*—Place the thumb over the mouth of the test tube and shake vigorously for five to ten seconds. Decant the water by holding a sterile wire gauze over the mouth of the test tube (after a little practice the water can be decanted without the use of the wire gauze). Add more water, shake, and decant as before. Repeat this process *ten* times. The object is still further to get rid of microbes clinging to the exterior of the nodules.

6. *Sterilizing the exterior of the nodules.*—After the last rinsing, described in the previous section, add to the test tube, in the place of water, a 5 per cent. carbolic acid or formalin solution and shake vigorously for eight seconds and decant the disinfectant immediately. The object is to kill microbes which may still be present upon the exterior and in the cork tissue and epidermal cells of the nodules. Naturally the antiseptic must be used quickly to prevent it from entering the interior of the nodule and killing the rhizobia themselves. Of these two disinfectants I am inclined to favor the carbolic acid, as it penetrates tissues less readily.

7. *Removing the disinfectant.*—This must be done at once. Rinse in boiled water (as in section 5) *five* times in rapid succession, to get rid of all traces of the disinfectant. The importance of this is evident, for should any considerable trace of the disinfectant remain it would destroy the rhizobia in the next process. No tests have been made to determine what the resisting power of rhizobia is to these disinfectants. I am inclined to believe that they have considerable resisting power, as is evidenced by their behavior in acid culture

media. Microbes differ widely in this regard. For example, typhoid fever germs and others, survive in a 0.5 per cent. carbolic acid solution.

8. *Crushing the nodules.*—Decant the last supply of water and transfer the tubercles to a thoroughly sterilized watch glass or similar small container (small salt cellar does well), and crush the tubercles with a sterile solid glass rod with rounded end. The crushed tubercles with the moisture that remained from the last rinsing form a pulpy mass. Cover with a sterile cover, such as a glass plate or a watch crystal, and set aside until ready to make the isolation cultures. If some time is required, the covered crush preparation should be placed upon a sterilized portion of the table and covered with a sterilized beaker or bell jar to present contamination by means of air microbes.

9. *Preparations for isolating rhizobia.*—It is assumed that the desired culture media have been prepared in the usual way. I would advise using the usual very slightly alkaline solid media of beef extract, salt, gelatin, and agar. Only sufficient agar should be used to give it solidity (about 1.5 per cent.). A number of test tubes about one-half to two-thirds full of the culture medium, sterilized by the fractional method, should be on hand ready for use. Six of these tubes should be placed in the steam sterilizer so that the medium may be thoroughty liquefied. This will require half an hour or more. They should be placed in the sterilizer about the time that the work of cleaning the tubercles is begun. Three thoroughly sterilized Petri dishes should be on hand ready for use. Number these one, two, and three.

10. *Preparing the test tubes for inoculation.*—When the culture medium in the test tubes is thoroughly liquefied, place the tubes into a beaker of moderately cool water. As soon as the water becomes hot from the heat of the tubes, replace it with fresh moderately cool water. While the tubes are still quite warm to the touch, place them in a beaker of water having a temperature of about 50° C., the object being to keep the medium liquefied. In this considerable care is necessary. The medium must not be too hot, nor should any portion of it be allowed to coagulate. The tubes should feel warm (not hot) to the touch when used. The tubes can be cooled

much more rapidly by holding them under a faucet of running water, but this is not recommended to the beginner because of the danger of cooling the tubes too much. Number four of the tubes and add two with blank labels for reserve purposes in case of accident or special requirement.

11. *Preparations for making the inoculations.*—A lighted Bunsen burner and a platinum wire loop fastened into a glass rod are all that is required. Wipe the table on which the work is to be done with a cloth moistened in some disinfecting solution.

12. *Inoculating tube no. 1; first attenuation.*—Heat the platinum loop in the Bunsen flame, allow it to cool and dip into the root nodule crush preparation, being careful not to include larger portions of tubercles, and introduce the loopful into test tube no. 1, shaking the platinum loop back and forth a few times within the medium. Replace the cotton plug in the tube, heat the loop in the Bunsen flame to a red glow, and place to one side. Now roll the test tube, kept in vertical position, between the hands to mix thoroughly the contents. Roll for five or six seconds.

13. *Inoculating tube no. 2; second attenuation.*—Take two loopfuls out of tube no. 1 and transfer them to tube no. 2; sterilize needle flames as before, and roll tube no. 2 as above.

14. *Inoculating tube no. 3; third attenuation.*—Take two or three loopfuls out of tube no. 2 and transfer to tube no. 3 and proceed as under section 12.

15. *Inoculating tube no. 4; fourth attenuation.*—Take three loopfuls out of tube no. 3 and transfer to tube no. 4 and proceed as under section 12.

Immediately after rolling, the tubes are to be replaced in the beaker of warm water to prevent the media from coagulating. Close application to detail is very important in order to avoid confusion. It is well to rehearse mentally the different manipulations before beginning with the tube inoculations or attenuations.

16. *Plating contents of inoculated tubes.*—Tube no. 1 may be rejected, but it is preferable to set it aside for control observations. Roughly estimated it contains several thousand rhizobia quite evenly distributed. Pour contents of tube no. 2 into Petri dish no. 1 which has been kept in a warm place (about 50° C.), spread the medium

evenly by inclining the dish slightly from side to side and set aside to cool. Pour contents of tube no. 3 into Petri dish no. 2, and contents of tube no. 4 into dish no. 3, spread medium and set aside. After the media in the Petri dishes are well coagulated, place the dishes where they will not be interfered with. They may be placed in an incubator provided the temperature is kept well below the melting point of the culture medium. I have obtained the most uniform results by keeping the plate cultures at the normal temperature of the laboratory.

About the third day the growths will begin to appear as very small light grayish specks; several hundred, more or less, in dish no. 1; twenty-five to thirty in dish no. 2; and perhaps only five to ten in dish no. 3. These are rhizobia cultures, and if the work was well done there will in all probability be no foreign microbes present. The cultures on the upper surface of the medium will be circular in outline, while those within the medium will be spindle-shaped. Growth of cultures is comparatively slow, thus giving ample time for study and to make tube cultures, plate cultures, etc. Tube cultures have been quite fully described in previous papers, likewise the morphology of the rhizobia grown in various artificial media.

Various other methods of isolating rhizobia have been tried successfully, but the method just described is recommended as giving the best results. It is somewhat lengthy in detail, but nevertheless simple in operation. It will be found that the isolation and cultivation of rhizobia is indeed simple, and even the beginner in the study of bacteriology will wonder what might have been the cause of the difficulties encountered by the earlier (1886 and later) investigators of the root nodule organisms. The difficulties however will become somewhat evident on examining the cultures microscopically. The morphological characteristics are found to be entirely changed, so that there is no similarity between the organism as it appears in the nodule and the organism as it appears in the artificial culture media.

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San Francisco.

BRIEFER ARTICLES.

THE PHYSIOLOGICAL CONSTANTS OF PLANTS COMMONLY USED IN AMERICAN BOTANICAL LABORATORIES. I.

THE series of papers of which this is the first will give the results of careful study undertaken to find which of our common plants are best adapted for the demonstration of each of the physiological phenomena of plant life. The work is being done in the Laboratory of Plant Physiology at Smith College with the advice of Professor W. F. GANONG. The literature of the various subjects is of course being used; but citation will be omitted for the most part, because the work is all being done *de novo*, and for the sake of teachers rather than investigators. It is intended that the resultant data shall enable teachers of botany to select in each case the best plant for the particular experiment in hand, and to know quantitatively the physiological behavior of each of the common kinds. Since most of the teaching of botany in this country is necessarily done in the winter, when out-of-door plants are not available, only greenhouse plants have been considered; and of these for the most part only the more common kinds, which can be grown in any ordinary room. Exceptions have been made in a few cases where a less common plant has been found to be especially good.

I. CHLOROPHYLL SPECTRA.

In order to compare the spectra of chlorophyll from different plants, it is necessary to adopt some standard solution and some uniform method of examining the leaves. The optimum amount of leaf for giving a clear solution, without filtering, from most leaves was found to be $25^{\text{sq cm}}$ of the leaf in 15^{cc} of 95 per cent. ethyl alcohol, and accordingly I have taken this as the standard solution. A larger proportion of leaf gives a clouded solution, which must either be filtered or be cleared by adding more alcohol. Solutions may be made equally well with methyl or wood alcohol.

The solutions were made as follows: A beaker was fitted with a cork in which three holes were made to allow two test tubes, holding the solutions, and a thermometer to be suspended in the beaker. The beaker was filled with water and set on a tripod over a Bunsen burner. The optimum temperature for extraction of the chlorophyll was found to be 50–55° C., at which the water in the beaker was kept. A tin hood was set over the

beaker to exclude all light during the extraction of the chlorophyll. In order to eliminate individual peculiarities, each experiment was done in duplicate for two different plants of each species studied. The time required for extraction of the chlorophyll from leaves of different species varied widely and is recorded for each case in the table following. Except where otherwise stated, half-grown leaves were used, as in most cases they yield the best solution in the shortest time.

It was found that the transparency of living leaves was increased if the air was driven from the intercellular spaces and its place was taken by water. Accordingly in these experiments the leaves were put in water under the air pump for half an hour and examined at once. If they are allowed to stand in water for an hour, leaves like Pelargonium, Begonia, Oxalis, etc., show the characteristic spectrum of incipient decomposition of chlorophyll. In the table the spectrum of a single leaf is given in each case.

The spectra were observed by a Kohl spectroscope, Hoffman form, with a comparison prism; one which is ample for all educational work. The Welsbach light was used.

As is well known, chlorophyll when examined by means of a spectroscope shows in all seven distinct absorption bands which are usually designated by the Roman numerals. I is a broad black band in the red; II is a narrow gray band in the red, near the orange; III is a gray band in the orange near the yellow; IV is a dark band in the middle of the green; V, VI, and VII are broad black bands in the blue, usually absorbing it to such a degree that with a few exceptions they can be seen separately only in a very thin layer of a dilute solution. These seven bands are never all present at one time.

In the following table the letter *d* means the band is dark; *f* that it is faint; *vf* that it is very faint; and *o* that it is absent. Where the spectrum of a living leaf differs from that of the chlorophyll solution its differences are indicated by the bracketed letters. The time required for the extraction of the chlorophyll is given in minutes. The thickness of the layer of the solution in each case was 15^{mm}. "Typical green" means the average unchanged color of chlorophyll.

In the table it will be seen that certain leaves are described as yielding a yellow-green solution. In all these cases the solution gives an acid reaction with litmus paper and the spectrum is of a distinct character from that of the other leaves. These facts, with others developed in the course of this study, show that we must distinguish between two distinct classes of chlorophyll spectra: the "normal chlorophyll spectrum" show-

Name of plant	Time in mins.	Color of solution	Bands						
			I	II	III	IV	V	VI	VII
Abutilon (golden bells) (young leaf)	5	typical green	d	d (o)	f	o	d	d	d
Asparagus Sprengeri	30	light green	d	vf	vf	o	o	d	d
Avena sativa (oats)	5	bright green	d	d	f	o	d	d	d
Begonia coccinea	2	yellow	d	o	o	f	d	d	d
Begonia coccinea	15	brown-yellow	d	d (f)	o (f)	d (o)	d	d	d
Cineraria cruenta	10	bright green	d	o (f)	o (f)	o	o	d	d
Cestrum elegans (young leaf)	5	dark green	d	f (vf)	f	o	d	d	d
Chrysanthemum frutescens	30	pale green	d	f (o)	o	o	d	d	d
Euphorbia pulcherrima (Poinsettia) (young leaf)	10	dark green	d	f (o)	f	o	d	d	d
Ficus elastica (rubber plant)	120	pale green	d	f	o	f	d	d	d
Ficus elastica (young leaf)	15	pale green	d	d (o)	f (o)	f (o)	d	d	d
Ficus repens (young leaves)	10	pale green	d	f (o)	f (o)	o	d	d	d
Fuchsia speciosa	15	yellow-green	d	o (f)	o (f)	f (o)	d	d	d
Hedera Helix (English ivy)	5	dark green	d	d (o)	f (d)	vf (o)	d	d	d
Heliotropium peruvianum (Heliotrope)	30	pale green	d	f (vf)	o (f)	o	d	d	d
Impatiens Sultani	15	bright green	d	f (o)	o (vf)	o	d	d	d
Jacobinia magnifica (young leaf)	30	dark green	d	f	f	o	d	d	d
Lycopersicum esculentum (tomato)	10	pale green	d	vf	vf (f)	o	d	d	d
Oxalis Bowiei	15	yellow-green	d	f	o (f)	d(o)f(o)	o (d)	d	d
Pelargonium domesticum	15	yellow-green	d	f	o (f)	d (o)	d	d	d
Pelargonium peltatum	15	green-yellow	d	f (o)	o (o)	d (o)	d	d	d
Pelargonium zonale	15	brown-yellow	d	f	o (f)	d (o)	d	d	d
Primula obconica	1	bright green	d	f (o)	f (vf)	o	d	d	d
Primula sinensis (Chinese primrose)	15	dark green	d	d (vf)	f	o	d	d	d
Primula verticillata	5	pale green	d	f (o)	f (vf)	o	d	d	d
Raphanus sativus (radish)	2	bright green	d	d (o)	f (vf)	o	d	d	d
Ricinus communis (castor bean)	15	pale green	d	f (vf)	f (vf)	o	d	d	d
Salvia involucrata	30	pale green	d	f (o)	f	o	d	d	d
Senecio mikanioides	15	pale green	d (f)	o	o	o	o	d(o)	d
Senecio Petasitis	15	typical green	d	f	f	o	d	d	d
Tropaeolum majus (nasturtium)	30	typical green	d (f)	o	o	o	d (o)	d (c)	d
Vicia Faba (horse bean)	2	bright green	d	f	f	o	d	d	d

ing bands I, II, III, V, VI, and VII, given by living leaves and by solutions made by extracting the chlorophyll quickly with only a minimum amount of heat; and the "decomposition chlorophyll spectrum," having bands I, II, IV, V, VI, and VII. This decomposition spectrum is given by solutions (*a*) from boiled leaves, (*b*) from leaves which contain acid salts (Oxalis, etc.), (*c*) by solutions made by prolonged immersion of leaves in cold alcohol, (*d*) by solutions in which the chlorophyll is decomposed by adding a drop of hydrochloric acid, or by exposure to direct sunlight for a few minutes, and (*e*) by living leaves after standing in water several hours.

On further decomposition of chlorophyll by light or acids an extra band appears in the green beyond IV and band V in the blue disappears. It is to be remembered that these spectra were given by standard solutions made as described above. With weaker solutions the bands become fainter, and with increasing diluteness bands II and III disappear.

Comparatively few living leaves show all the bands of the normal

chlorophyll spectrum in a single leaf, but all leaves give all these bands when two or three are used. The bands in the spectrum of a solution of chlorophyll were compared with those in the living leaf in two ways: (1) by observing the two spectra at the same time, one with direct light and the other by means of the comparison prism; (2) by observing the position of the bands in direct sunlight with reference to the Fraunhofer lines. In all cases the bands appear in exactly the same place in the spectrum of a leaf as in that of a standard solution giving the normal chlorophyll spectrum.

From the above it is plain that the most typical spectra are yielded by those non-acid leaves from which the chlorophyll can be extracted most easily, namely (in the order of their excellence) *Primula obconica*, *Raphanus sativus*, *Vicia Faba*, *Abutilon* (young leaf), *Avena sativa*, *Cestrum elegans* (young leaf), *Euphorbia pulcherrima* (young leaf), *Lycopersicum esculentum*, *Primula sinensis*, and *Ricinus communis*.

Another well-known optical property of chlorophyll is its fluorescence, all solutions of chlorophyll being slightly fluorescent. The products of the decomposition of chlorophyll, however, seem to be more fluorescent than chlorophyll itself. A solution of chlorophyll extracted with boiling alcohol from boiled leaves in the light is more fluorescent than a solution of unchanged chlorophyll. Of course some leaves are better than others, and of all the leaves in the above table, the very best are *Jacobinia magnifica*, *Cineraria cruenta* (the dark-leaved varieties), *Cestrum elegans*, and *Hedera Helix*. Standard solutions from these leaves give a brilliant blood-red fluorescence.—Sophia Eckerson, *Smith College, Northampton, Mass.*

FURTHER OBSERVATIONS ON THE STRUCTURE OF THE STARCH GRAIN.[1]

In a previous paper (1) on the structure of the starch grain, I showed that the peripheral layer of the potato starch grain breaks and recurves on treatment with certain reagents, much like the cutin layer of an epidermal cell on treatment with sulfuric acid. While I had previously observed that this peripheral layer is stained with certain of the aniline dyes, I thought that the effect might probably be due to the remains of plastids or protoplasmic material, rather than to the presence of a distinct membrane. I am now satisfied, however, that it can be demonstrated that the peripheral layer of the starch grain is a distinct membrane.

It is well known that upon the addition of an iodin solution to unaltered

[1] Read at the meeting of the Society for Plant Morphology and Physiology, December 1903.

starch grains at the ordinary temperature, the grains are colored blue; while the solution or filtrate remains colorless or some shade of yellow, depending upon the amount or strength of iodine solution that has been used. If on the other hand the grains are triturated with sand (one part of starch to five of sand) for a short time previous to the addition of the water, the filtrate will be colored a deep blue upon the addition of an iodin solution. Heretofore it has been supposed by some that the blue coloration was due to the presence of fine particles which went through the filter and were suspended in the filtrate, while in reality there is a true solution of the starch; or in other words the soluble starch has been liberated from the grain by the rupture of the peripheral layer. A polariscopic examination of the starch solutions, after clarification by means of talcum, showed that they have marked dextro-rotatory properties. The angle of rotation for solutions of corn, wheat, maranta, and potato starch ranged from 0.3814 to 0.8770°. Furthermore, a microscopic examination of the triturated starch shows the grains to be in various stages of disintegration, and this taken together with the behavior of starch toward iodin, as well as the other observations referred to, would indicate that the starch grain consists of a membrane that is insoluble in water at ordinary temperature; and an interior portion which is at least in part soluble in water at ordinary temperature, that is consists of soluble starch or starch that is colored blue with iodin. This technique enables us to differentiate at once the soluble starch present, which heretofore has not been supposed to exist in the unaltered starch grain. As to whether this substance is identical with the granulose of NÄGELI (**2**) is a problem which remains to be determined. It may be added that this method furnishes a means of separating the constituents in the unaltered grain, and is to be preferred to the methods which have been employed heretofore, of using solutions of ferments or mineral acids, which give rise to altered substances, and hence do not give a true differentiation of the substances as they naturally occur in the starch grain.

This brings us then to consider the views of RASPAIL (**3**), in connection with those of others, in regard to the nature of the starch grain, which may be summarized as follows:

1. The starch grain consists of a membrane which is insoluble in water, and a more or less soluble content, as pointed out by RASPAIL.

2. It develops from a centric or excentric point, to which layer after layer is added, a view first advanced by FRITSCHE (**4**), and subsequently enlarged upon by SCHIMPER (**5**), who demonstrated that its growth is dependent upon the function of the leucoplastids.

3. The content of the grain consists of at least two different substances, as first pointed out by NÄGELI (**2**), and later confirmed by MEYER (**6**), SCHIMPER (**5**), and others, who showed in addition that the structure might be compared to that of sphero-crystalloids.

THE ACTION OF IODIN UPON STARCH.

The effect of iodin upon starch has been the subject of considerable investigation and speculation by both botanists and chemists. MYLIUS (**7**) held to the view that the blue coloration formed on the addition of iodin to starch is due to the formation of a compound of hydriodic acid and starch. MEINEKE (**8**) also contended that hydriodic acid is essential to the formation of the blue color of an iodin-starch solution. STOCKS (**9**), SEYFERT (**10**), and ROUVIER (**11**) are of the opinion, however, that this colored compound does not contain hydriodic acid, the last named investigator having shown that it may be formed in the presence of alcohol as well as water, and all of them agreeing that a true chemical compound (iodid of starch) is formed.

On the other hand, KÜSTER (**12**) and MEYER (**6**) believe that the so-called iodid of starch is not a true chemical compound, but that the iodin and starch are combined in much the same way as the ingredients in an emulsion. This latter view has been more or less sanctioned on account of the statements in the textbooks that the iodin is removed from the combination on treatment with carbon disulfid. My observations, however, show that if the starch is in excess of the iodin (using 1^{gm} of potato starch and 10^{cc} of a solution containing 0.1 per cent. of iodin and 0.5 per cent. of potassium iodid), or if the iodin and starch be in such proportion that all of the free iodin is taken up, and the mixture is dried before adding the carbon disulfid, even on allowing it to act for six months, the carbon disulfid is not colored; that is, does not take up the iodin, the same being true of alcohol and chloroform. This result, taken together with the previous experiments in the treatment of starch with sand and then with iodin, would seem to confirm the view that a true chemical compound is produced by iodin and soluble starch, whether in the grain or in solution.

That the amount of soluble starch varies in the starch grains of different origin can be demonstrated by the treatment of definite amounts of the starches with definite quantities of iodin solution. On treating 0.5^{gm} of the several commercial starches with 2^{cc} of iodin solution (0.1 per cent. of iodin and 0.5 per cent. of potassium iodid), it will be found that there is a marked difference in the intensity of the color in the grains, those of potato and maranta being colored deep blue, while those of corn and wheat

are scarcely at all colored, the mixtures being somewhat of a purplish color. These observations seem to accord with the experiments of ROUVIER (**13**), who found that the different classes of starch took up varying amounts of iodin, and are contrary to the statement by STOCKS (**9**) that the shade of color varies with the amount of iodin but not with the different starches. In other words, the shade and intensity of color not only vary according to the strength of iodin solution, but also according to the kind of starch used.

It is well known that if a starch solution be treated with iodin in the cold, a blue coloration will be the result, and that if this solution be then heated the blue color will disappear, but will return on cooling the solution, though less intensely than before. This peculiar behavior of starch and iodin has never been satisfactorily explained. NÄGELI and SCHWENDENER (**14**) consider that the loss of color on the application of heat is due to the production of hydriodic acid; but this does not explain the reappearance of the color on cooling; and so far as I am aware no one has ever demonstrated the presence of this acid. If this acid were present, then according to MYLIUS (**7**) and MEINEKE (**8**), the blue color would remain on heating the solution, since they claim, as already pointed out, that the blue color is due to the presence of this acid. It is thus apparent that there is considerable contradiction in the arguments that have been presented on this subject.

The following experiments tend to explain this phenomenon. If we take 0.5^{gm} of potato starch and mix it with 50^{cc} of water and heat the mixture in an Erlenmeyer flask of about 100^{cc} capacity on a water bath to a temperature of about 80° C. for one hour, and then add 5^{cc} of iodin solution (iodin 0.1 per cent. and potassium iodid 0.5 per cent.) and place a piece of ordinary filter paper, which usually contains starch, over the top of the flask, and then continue the heating until the solution is decolorized, which takes place at about 80° C., the filter paper will be colored blue, showing that some of the iodin has been volatilized. Furthermore, on cooling the solution, as already pointed out, the blue color begins to return at about 65° C. and increases in intensity until at about 40° C. it reaches the maximum; though it is less intense than the color produced when an iodin solution is added to a starch solution cooled to this temperature. These results may be interpreted as showing that iodin forms a compound with starch which is dissociated on the application of heat; that is, at a temperature between 60 and 80° C., part of the freed iodin is volatilized as such, and the amount remaining in solution depends upon the length of time the heat is applied. Also the loss of iodin in this way accounts for the decrease in color of the starch-iodin solution on cooling.

An attempt was made to remove the free iodin from the hot colorless starch-iodin solution by the addition of chloroform, but it was found difficult to handle the material, as chloroform boils at about 60° C. Subsequent experiments, however, showed that unaltered potato starch grains as well as a solution of potato starch would remove the iodin from a chloroformic solution at the ordinary temperature. These experiments were conducted as follows: 5^{cc} of an iodin solution, of the strength already stated, were shaken in a separatory funnel with 25^{cc} of chloroform, and most of the chloroform containing the iodin separated. To this was added 5^{gm} of dried starch. The latter, however, did not take up the iodin from the solution; but on the addition of a small quantity of water (about 10^{cc}) and after shaking the mixture, the starch and iodin combined, and almost all of the iodin in the chloroformic solution may be removed in this way. The addition of 2^{cc} of potassium iodid solution (5 per cent.), even with 0.5^{gm} of starch, causes the iodin to be taken up immediately. The starch in a soluble starch solution also combines immediately with the iodin in a chloroformic solution.

In summing up the observations on the behavior of iodin and starch, it seems to me that we are dealing with a chemical compound of iodin and soluble starch; but that the combination is a feeble one, being easily dissociated upon the application of heat, and the iodin being more or less volatilized. Also the facility with which soluble starch takes up the iodin in a chloroformic solution indicates that the affinity of starch for iodin is considerably greater than heretofore supposed.

STAINING OF THE GRAIN.

While I have obtained at times some beautiful results by the use of anilin stains, it has been impossible for me until now to duplicate some of my earlier results. I have succeeded, however, in developing a method in the staining of wheat starch which yields uniformly satisfactory results. It is as follows: to 0.500^{gm} of wheat starch add 2^{cc} of an aqueous iodin solution (containing 0.1 per cent. of iodin and 0.5 per cent. of potassium iodid); mix well and allow the mixture to stand from 20 to 30 minutes in a porcelain dish or watch crystal; then add 2^{cc} of a saturated aqueous solution of gentian-violet (1^{gm} of gentian violet to 100^{cc} of water); allow this to stand from 12 to 24 hours, examining the grains from time to time by mounting them in water. When the grains are satisfactorily stained, the mixture is transferred to a filter and the excess of stain is removed as quickly as possible by washing the magma with water. The material is then allowed to dry spontaneously or between pieces of bibulous paper.

For examination it is then mounted in Canada balsam, the preparation being permanent for years, as is also the case with the unmounted material.

Corn starch may also be stained by the use of this method, but in the staining of potato and maranta starches it is necessary to use weaker iodin solutions. I have not evolved entirely satisfactory methods, however, for uniformly staining these latter starches.

It may be of interest to state that the foregoing method has certain features which are similar to those of the gentian-violet method used in demonstrating the so-called continuity of protoplasm in the vegetable cell wall (**15**). In the study of the continuity of protoplasm a swelling reagent, such as sulfuric acid, is used and a comparatively short time is consumed in the whole operation; whereas in the method proposed for the staining of wheat starch the water used may be considered a swelling agent acting on the grain during a longer time. The analogy in the results are so striking that students who are interested in the study of the continuity of protoplasm will do well to compare their results on the cell wall with those obtained in the study of the wheat starch grain by the method just described.

The author acknowledges his indebtedness to Miss FLORENCE YAPLE for valuable assistance in the preparation of this paper.—HENRY KRAEMER, *Philadelphia College of Pharmacy*.

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CURRENT LITERATURE.

BOOK REVIEWS.

The water-lilies

THE CARNEGIE INSTITUTION has published a sumptuous volume bearing the above title.[1] Mr. CONARD, the author, is a Senior Fellow in Botany at the University of Pennsylvania, and has spent four years in the preparation of this monograph. The purpose seems to be to present water-lilies from every botanical standpoint; and so far as this can be done by one man making most diligent use of his time it has been well done. The conception that research is the exhaustive study of a single form, and that all observations should be reported whether pertinent to anything or not, is well exemplified in this volume. The diligence it has demanded is beyond praise; the ideas directing it are questionable. The following statement in the preface is significant: "had the learned doctor (CASPARY) of Königsberg assembled his vast knowledge into one connected whole, the present work would be needed chiefly as a translation." As this implies, the volume is the assembling of a vast amount of information about water-lilies; and the scope of it is expressed by the following statement: "It has therefore seemed important to bring together the knowledge of the genus in all of its botanical relations and in its bearings on human life and history."

It is questionable whether any one man is equipped to do this as a contribution; he may do it as a compilation. Just here is the vital difference between research and collected information. It is unfortunate that many who are directing research do not make the distinction. It would reduce publications in bulk and save an immense amount of time consumed in discovering the contribution. In the present work, for example, there have doubtless been made some real and valuable contributions to botany, but there is no way of discovering them without looking through nearly three hundred large pages.

There are eight parts in the volume, each presenting water-lilies from a distinctly different point of view, as follows: (1) history, including oriental literature as well as pre-Linnaean literature; (2) structure, which is for the most part anatomy; (3) development, by which is meant what is usually considered under morphology; (4) physiology; (5) taxonomy; (6) distribution; (7) hybrids and garden varieties; and (8) culture and uses. The taxonomy must have been in a fairly good condition, except perhaps as to nomenclature, for of the thirty-four species recognized only one is described by the author as new.

The thirty plates are works of art, twelve of them being colored. It is a gratification to know that the Carnegie Institution has money enough to spend in this lavish way.—J. M. C.

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[1] CONARD, HENRY S., The water-lilies, a monograph of the genus Nymphaea. 4to. pp. xiii + 279. *pls.* 30. *figs.* 82. The Carnegie Institution of Washington. 1905.

French instruction in botany

BONNIER and SABLON[2] have published the first volume of a text-book of botany for the use of classes in universities, and in schools of medicine, pharmacy, and agriculture. The first impression is that of great bulk, and it is almost beyond belief that such an amount of material can be absorbed by undergraduate students in a continuous course. The authors, however, are teachers of large experience, and must know what the French situation demands and how much the French student can endure. The illustrations are for the most part excellent, and are said to be published in this volume for the first time. This means a large stock of new illustrations of well-known structures; and this stock really constitutes the chief contribution of the volume.

The text is clear and well organized; and the distinct paragraphing is all that could be desired in a text for elementary students. The material is brought together from every direction, making the volume a compendium of information concerning the topics presented. However, it gives an impression of voluminousness and diffuseness rather than of logical and compact presentation.

The four parts of this first volume are very unequal. The first part (138 pp.) consists of a general introduction, beginning with the characteristics of living things and gradually approaching plants. The second part (602 pp.) presents the morphology of angiosperms, under the following topics: leaf, stem, root, flower, and development. In this part the emphasis is laid upon anatomy and what one may call for convenience the older morphology.

The third part (524 pp.) deals with the families of angiosperms, that dreariest of all wastes for the elementary student, but perhaps demanded by the French schools. Just what is done with this part of European text-books has always been a mystery to the majority of American teachers. The fourth part (62 pp.) presents the gymnosperms and closes the volume.

A noticeable feature of the presentation is the singular blindness to published work. In an elementary text this seems natural, and usually would occasion no remark; but this large compendium cites literature, and further dignifies the citations by collecting them in a list at the end of each part. The four lists include 108 titles; and when they are examined, it is evident that the selection has been at random, without reference to the importance of the papers or to the representative character of the lists. In fact, the impression upon students and colleagues would be far better if no citations had been attempted.

Taking the book as fairly representative of botanical instruction in French schools, one may conclude that the instruction includes more of the old botany than the new; presents a mass of details rather than general organizing ideas; and calls for diligence and a good memory rather than for initiative.—J. M. C.

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[2] BONNIER, GASTON, et SABLON, LECLERC DU, Cours de botanique. I. Phanérogames. 8vo. pp. iv+1328. *figs. 2389*. Paris: Librairie générale de l'enseignement. 1905.

MINOR NOTICES.

A SECOND REPORT to the Evolution Committee of the Royal Society has been made by BATESON, SAUNDERS, PUNNETT, and HURST,[3] under the subtitle "Experimental studies in the physiology of heredity." About two-thirds of this report deals with plants and the rest with poultry. The plants used were Datura, Matthiola, *Salvia Horminum*, *Ranunculus arvensis*, Pisum, and *Lathyrus odoratus*, the most attention being given to Matthiola, Pisum, and Lathyrus. The numerous experiments present too many important details to permit of adequate review, but several features deserve special mention. In Matthiola it is found that two races which are constantly glabrous when pure-bred may, on crossing, produce hoary canescent offspring, and that in certain combinations this hoariness is coupled with purple flower-color, both the hoariness and the purple color being recognizable as atavistic characters. These reversions occur invariably when cream or white-flowered glabrous stocks are crossed with those of any other color; but the various sap-colors (e. g., purple, flesh, red, copper, etc.) crossed with each other conform strictly to Mendelian expectation in regard to hoariness, though with respect to color there is a complication introduced by the presence of the atavistic purple in addition to the two parental types. Very similar reversions and half-reversions are also found regularly in sweet peas. Generally white was found recessive to all sap-colors, and cream recessive to both white and the sap-colors, so that cream-colored sweet peas are always homozygous and can produce nothing but cream-colored offspring. With one exception, white crossed with any sap-color gave reversion in the first generation to purple or to "painted lady" (red bicolor). This occurrence of two reversionary types gives rise to complications which have not yet been well worked out.

Perhaps the most novel result is seen in the different behavior of two white-flowered strains known to the trade as "Emily Henderson" and distinguishable from each other only by the form of the pollen which is either long or round. The long pollen is generally characteristic of the various strains of sweet peas, while the round appears to be limited to this one strain of "Emily Henderson," and to the various dwarf sweet peas or "Cupids" which are believed to have sprung from it. When any pure-bred white-flowered strain having long pollen was crossed with any colored strain the first-generation hybrids were always purple. When white with round pollen was crossed with blue sap-colored, it likewise produced F_1, but when crossed with red sap-colored the first generation was always painted lady. When pure-bred white "long" was crossed with pure-bred white "round," the first generation hybrids were sometimes purple, sometimes painted lady. If, on the other hand, *extracted* whites are used, whether they have long or round pollen, no reversion takes place when they are crossed together, and F are all white-flowered.

[3] BATESON, W., SAUNDERS, Miss E. R., PUNNETT, R. C., and HURST, C. C., Reports to the Evolution Committee of the Royal Society. Report II. Experimental studies in the physiology of heredity. 8vo. pp. 154. London: Harrison & Sons. 1905.

...COLORADO...
STATE NORMAL SCHOOL

This difference of behavior between pure-bred and extracted whites is suggestive of CASTLE'S[4] recently reported results with guinea-pigs, in which several white individuals showed the presence of latent black pigment by transmitting it to a certain proportion of their offspring. The whites extracted from the non-black members of these crosses proved to be pure albinos incapable of producing black offspring.—G. H. SHULL.

MACDOUGAL[5] has published, with the cooperation of A. M. VAIL, G. H. SHULL, and J. K. SMALL, some of the results of the study of Oenothera that have been made at the New York Botanical Garden. Thus far the attempt to find *O. Lamarckiana* in its native state has been unsuccessful, although herbarium and other records make it "fairly conclusive that it is a true and independent species, native to America." That this species has remained unchanged for over a hundred years is certain. *O. grandiflora*, *O. Lamarckiana*, and *O. argillicola* are more closely related to one another than any of them are to *O. biennis*. An expedition by S. M. TRACY undertaken for the purpose of the rediscovery of *O. grandiflora* was successful. *O. biennis*, as commonly understood, includes two or more elementary species, and *O. cruciata* embraces three such species. These facts make it evident that the resolution of a "species" into its elementary species is the first requisite for the study of mutation, casual field observations being almost worthless. Careful studies were made of hybrids of *O. Lamarckiana* with *O. cruciata* and *O. biennis*. The occurrence of known mutants, as *O. rubrinervis*, *O. albida*, *O. gigas*, etc., from a culture of *O. Lamarckiana* was observed, showing that the mutative period of the latter is still present, and that the same mutants may occur in diverse environments. Some hitherto unknown mutants were also observed. Results of statistical studies are set forth in some detail; while the mutants show great variability, there is yet a great gap between them and the parent form. A summary of this paper[6] is given in another place. —H. C. COWLES.

WHITE[7] has published a preliminary report on the Hymeniales of Connecticut, illustrated by half-tone reproductions of excellent photographs. The purpose of the report is to compile, so far as possible, a complete list of native species, with notes as to the characteristics of the genera.—J. M. C.

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[4] CASTLE, W. E., Heredity of coat characters in guinea-pigs and rabbits. Papers of the Station for Experimental Evolution at Cold Spring Harbor, New York, no. 1. pp. 78, *pls. 6*. Washington: Carnegie Institution of Washington.

[5] MACDOUGAL, D. T., VAIL, A. M., SHULL, G. H., and SMALL, J. K., Mutants and hybrids of the Oenotheras. Pp. 57. *figs. 13. pls. 22*. Carnegie Institution of Washington, Publication No. 24. Washington, 1905.

[6] MACDOUGAL, D. T., Studies in organic evolution. Jour. N. Y. Bot. Gard **6**:27–36. 1905.

[7] WHITE, EDWARD ALBERT, A preliminary report on the Hymeniales of Connecticut. State Geol. and Nat. Hist. Survey, Bull. 3. pp. 81. *pls. 40*. 1905.

CLINTON[8] has described the fifty species of smuts known to occur in Connecticut, introducing the descriptions by a general account of the characters of the group. Numerous drawings and reproductions of photographs illustrate the paper.—J. M. C.

SCHAFFNER[9] and his associates have made an ecological study of a glacial lake near Columbus, Ohio. Many of the typical bog plants, such as sphagnum, are absent; but some, as Decodon, are present in abundance.—H. C. COWLES.

OSGOOD,[10] in an account of a reconnoissance in Alaska mainly concerned with birds and mammals, gives notes on the distribution of the more characteristic plants that will interest plant geographers.—H. C. COWLES.

NOTES FOR STUDENTS.

FAULL[11] has made a cytological investigation of the ascus, studying the origin of the asci from the ascogenous hyphae, and their nuclear divisions and spore formations in a number of hitherto uninvestigated Ascomycetes, particularly *Hydnobolites* sp., *Neotiella albocincta*, and *Sordaria finicola*. He has examined thirty-six species in order to determine how the ascus originates from the ascogenous hyphae. He finds numerous cases in which the ascus does not arise from the penultimate cell of the recurved tip of an ascogenous hypha, as described for various Discomycetes but not for mildews. MAIRE and GUILLIERMOND have fully described deviations from this type, and FAULL in his examination finds that such is invariably the case in only eleven species. The ascus may bud out from the penultimate cell, although occasionally the septum between it and the terminal cell is lacking. The absence of this wall cutting off a uninucleate terminal cell at the tip seems to be the most frequent departure from the conventional type, being well illustrated by *Genea hispidula*, in which form the wall is always wanting. In some forms the asci arise from the terminal cell of the ascogenous hyphae and in others apparently from any cell. In every case definitely determined, the uninucleate stage of the ascus arises by fusion of two nuclei, which may be daughter nuclei or sister nuclei, either before or after entering the ascus. Extranuclear granules, staining like nucleoli and evidently nutritive in character, were observed in the neighborhood of nuclei in the asci. The constant appearance of these bodies is a characteristic feature at different stages. Similar bodies were also observed in ripe spores.

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[8] CLINTON, GEORGE PERKINS, The Ustilagineae, or smuts, of Connecticut. State Geol. and Nat. Hist. Survey, Bull. 5. pp. 45. *figs.* 55. 1905.

[9] SCHAFFNER, J. H., JENNINGS, O. E., and TYLER, F. J., Ecological study of Brush Lake. Proc. Ohio State Acad. Sci. **4**:151–165. 1904.

[10] OSGOOD, W. H., A biological reconnoissance of the base of the Alaska Peninsula. Pp. 86. *pls.* 5. *maps* 2. North American Fauna No. 24. Washington. 1904.

[11] FAULL, J. H., Development of ascus and spore formation in Ascomycetes. Proc. Bos. Soc. Nat. Hist. **32**:77–113. *pls.* 7–11. 1905.

This author believes that the spindles are of intranuclear origin, while the centrosomes and asters with which they are associated are of extranuclear origin. The nucleus occupies the dense cytoplasm which becomes differentiated about it. In Neotiella and Sordaria the protoplasm about the exceedingly large nucleus streams out irregularly into the foamy cytoplasm above and below; while the nucleus of Hydnobolites is surrounded by a hyaline area possessing a radiate structure. The fibers of the broad spindle taper in Neotiella and Sordaria to terminate in two very minute centrosomes, from which radiate very fine rays, often so fine as not to be easily demonstrable. The astral rays in Hydnobolites are long and coarse and easily observed. These rays stain differently from the centrosome, and there is no evidence that they are outgrowths from or that they are absorbed by the centrosome at the time of their disappearance. In Hydnobolites the chromosomes are very small, while those of Neotiella are large horseshoe-shaped bodies. The number of chromosomes may vary in different species, being four or five in Hydnobolites and six or seven in Neotiella. The method of spore formation is particularly interesting, as it does not at all correspond with that described by HARPER. A plasma membrane is organized about the sporeplasm before the nuclei pass into a resting condition. This membrane is formed entirely distinct from the astral rays, which do not appear to enter into its composition. The long thick astral rays of Hydnobolites change position, but so as to be thrown farther apart. A fusion is an impossibility. These rays may be seen distinctly even after the spores are delimited. The sporeplasm is delimited from the rest of the cytoplasm by the differentiation of a certain hyaline finely granular area. This specialized hyaline layer of protoplasm begins just outside the centrosome and proceeds progressively until it entirely encloses the sporeplasm. A plasma membrane is subsequently formed from or in this limiting area. Concurrently with this first membrane a second membrane is formed in contact with the first, which lines the cavity in which the spore is to lie. FAULL suggests that these membranes may arise by a cleavage in the limiting area, caused by its increased growth and differentiation and a pull on the part of the nucleus. Both plasma membranes are intimately concerned in laying down the spore walls between the opposed membranes. The time of the formation of the spore walls is variable in the different species and bears no relation to the delimitation of the sporeplasm. Multinucleate spores are usually septate, but those of Sordaria are unseptate. The multinucleate condition arises, at least in Sordaria, by karyokinetic division of the nucleus of the spore. Where a septum is formed cutting off an enucleated portion, as the tail of the Podosporas, its organization is due to the direct action of the nucleus on the cytoplasm, since septa are formed only in the immediate neighborhood of nuclei. The author favors the view that homologizes the ascus with a zoosporangium of the Oomycetes, as an argument in favor of the origin of the Ascomycetes from the Oomycetes. He does not believe that the difference between the method of spore formation in the ascus and sporangium is so great as to prevent an assumption of their homology.—J. B. OVERTON.

WASIELEWSKI[12] has undertaken by theory and by his own experimental data to dispel what he calls the "Mitosendogma" of HEGLER. This so-called dogma consists in a discrimination between mitosis and amitosis, in which the former is regarded as the only process by which nuclear division can be accomplished and the potential qualities retained. According to this view, fragmentation of the nucleus is held without exception to involve loss of regeneration capacity. According to the author this dogma ignores the fact that amitosis is just as normal for many lower organisms as mitosis is for the higher. Further, the idea that the nucleus is the bearer of the hereditary qualities is a theory only, though so widely accepted as to be regarded often as a fact. A study of the influence of chloral hydrate, especially, on nuclear and cell division leads the author to conclude that both may occur amitotically in higher plants. This tendency to amitosis apparently dormant may be aroused by stimulants. Degeneration as a consequence of amitosis was not observed, and cells so divided can resume mitotic divisions without loss of capacity for development. Two modes of amitotic nuclear divisions were observed: *Diatmese* (dissection) and *Diaspase* (distraction). Thus, the latter are regarded as members in a phylogenetic series which includes mitosis and from which amitosis does not fundamentally differ. In the second section the author goes so far as to state that a given nucleus may begin its division mitotically and complete it amitotically. The physiological equivalence of mitosis and amitosis is advocated. NĚMEC'S paper, in which the author is believed to have confused amitosis with nuclear fusion, appeared after this second section had gone to press. The author promises a paper in which this matter will be considered.—RAYMOND H. POND.

A RECENT PAPER by LONGO[13] describes the nutrition of the embryo sac in the Cucurbitaceae, especially in *Cucurbita Pepo*. The principal point of interest is the behavior of the pollen tube, which, after discharging the usual function connected with fecundation, serves as an organ of food absorption. About the time the pollen tube reaches the embryo sac, an enlargement occurs in it a short distance from its extremity. After fertilization, slender branch-like outgrowths proceed from the enlargement and grow along the nucellus to enter a specialized region of the outer integument. This region is composed of cells containing food material, as starch, which is extracted by the haustorial prolongations of the pollen tube. In the meantime the epidermis of the nucellus, or its outermost layer of cells, becomes cuticularized and the cells near the chalaza become suberized. Thus it would seem that the path of the food material is through the vascular bundle of the outer integument to the nutritive tissue, and from thence to the embryo through the haustorial prolongations and the pollen tube. The remainder of the paper is occupied (1) with an account of the changes that occur

[12] WASIELEWSKI, W. VON, Theoretische und experimentelle Beiträge zur Kenntniss der Amitose. Jahrb. Wiss. Bot. **38**:377–420; **39**:581–606. *figs. 10.* 1904.

[13] LONGO, BIAGIO, Osservazioni e ricerche sulla nutrizione dell'embrione vegetale. Annali Botanica **2**:373–396. 1905.

in the endosperm and integuments during seed development; and (2) with reviews of various papers dealing with different methods of embryo sac nutrition. While LONGO's paper was in press, that of KIRKWOOD[14] appeared. A footnote by LONGO states that his observations and KIRKWOOD's do not agree in respect to the presence of a micropyle and the passage of the pollen tube through it in *Cucurbito Pepo*. LONGO maintains that in this species no micropylar canal is present, but that the pollen tube grows between the cells of the nucellus. As a micropyle is present in other species, he thinks KIRKWOOD has made a mistake in determination.—F. H. BILLINGS.

TWO INVESTIGATORS have published preliminary announcements of the results of a study of fertilization and the associated structures in *Juniperus communis*. NORÉN[15] says that during the summer following pollination the pollen tube grows into the tissues of the nucellus, but fertilization does not occur until the following year. The two male cells are equal in size. A ventral canal nucleus is formed, but it is not separated from the egg by a wall. The male cell is still surrounded by its cytoplasm when it enters the egg, but slips out from it as the sex nuclei come into contact. There are eight free nuclei in the proembryo before walls begin to be formed.

SLUDSKY'S[16] announcement was hastened by that of NORÉN. He reports that the entire development of the sexual generation, from pollen to fertilization, and from megaspore to embryo, lasts only one summer; the growth of the pollen tube lasting only two to six weeks. A ventral canal nucleus is formed, but disappears before fertilization. Centers with radiations are prominent in the egg; and are caused by the diminishing pressure which accompanies the formation of vacuoles. There are never more than two male cells in a pollen tube. The multicellular complex described for Cupressus by JUEL is regarded as due to abnormal material. Not more than two male cells ever enter the egg, and only one functions in fertilization. The nucleus of the male cell is still surrounded by its cytoplasm after it enters the egg. During fertilization there can be seen in the upper part of the egg the tube nucleus, neck cells, and even cells of the overlying tissue. In regard to the fusion of sex nuclei, the author agrees with NORÉN, and in regard to the embryo he agrees with STRASBURGER.—C. J. CHAMBERLAIN.

LILIENFELD[17] ascribes the indecisive results obtained by NEWCOMBE and RHODES in their study of the chemotropism of roots to inadequate methods. Among the sources of error unprovided for by them the author mentions (1)

[14] KIRKWOOD, I. E., The comparative embryology of the Cucurbitaceae. Rev. in BOT. GAZETTE **39**:73. 1905.

[15] NORÉN, C. O., Ueber Befruchtung bei *Juniperus communis*. Vorläufige Mitteilung. Arkiv. Bot. Svensk. Vetens. Akad. **3**: pp. 11. 1904.

[16] SLUDSKY, N., Ueber die Entwickelungsgeschichte des *Juniperus communis*. Vorläufige Mitteilung. Ber. Deutsch. Bot. Gesells. **23**:212–216. *pl. 6*. 1905.

[17] LILIENFELD, M., Ueber den Chemotropismus der Wurzel. Ber. Deutsch. Bot. Gesells. **23**:91–96. 1905.

traumatic disturbance due to resistance offered by gelatin surface to entering root; (2) positive aerotropism because of the stratum of air between the gelatin blocks; (3) diffusion of stimulating substances from one block to the other. In the author's improved method, only one large circular block of gelatin is used. After a cavity is made in the center of the block, the seedlings are planted in the gelatin at varying distances from the margin of the cavity, and into the latter the stimulating substance is then placed. By using this method negative responses were obtained in cases corresponding to which positive responses were obtained with the method of NEWCOMBE and RHODES. The former responses are regarded as chemotropic, while the latter are considered traumatropic.—RAYMOND H. POND.

THE GREATEST GAP in our knowledge of the morphology of Coniferales is in connection with the Araucarineae. THOMSON,[18] whose interesting work on the megaspore-membrane of gymnosperms has been noted, has published a preliminary statement of the results of his investigation of the tribe. The conspicuous features are the supernumerary nuclei found in the pollen tube, in one case reaching thirty in number; the failure of the pollen grains to reach the micropyle, lodging at the distal end of the scale and sending out their tubes from that point; the unusual freedom of the nucellus from the integument; and the peculiar arrangement and development of the archegonia, not described in this notice. The anatomical details also indicate a peculiar isolation of the tribe among Coniferales. The forthcoming monograph will be looked for with great interest. —J. M. C.

SCOTT[19] has discovered the sporangia of *Stauropteris Oldhamia*, a common plant of the English Coal-measures, which has been regarded as a much branched and naked rachis of a fern leaf. The ultimate branchlets are exceedingly numerous and slender, "occurring in dense, faggot-like groups." SCOTT now finds that these branchlets bore terminal sporangia of the ordinary fern type, except that there is a terminal stomium and no annulus. There is a suspicion that these may be the microsporangia of a pteridosperm, especially since the ovules of that group, so far as found attached, are also terminal upon ultimate branchlets. Another suggestion would be that such a position of sporangia attained among true ferns accounts for its occurrence among pteridosperms.—J. M. C.

IN A LIST of some unrecorded stations for New Zealand plants, COCKAYNE[20] includes *Carex Darwinii urolepis*, a plant hitherto recorded as occurring only in Patagonia, thus adding another form common to the floras of South America and New Zealand.—J. M. C.

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[18] THOMSON, R. B., Preliminary note on the Araucarineae. Science N. S. **22**:88. 1905.

[19] SCOTT, D. H., The sporangia of *Stauropteris Oldhamia* Binney. New Phytol. **4**:114–120. *figs. 2.* 1905.

[20] COCKAYNE, L., Some hitherto-unrecorded plant habitats. Trans. N. Z. Inst. **37**:361–367. 1905.

NEWS.

PROFESSOR EDUARD TANGL, University of Czernowitz, died recently at the age of fifty-seven years.

PROFESSOR LEO ERRERA, director of the Botanical Institute of the University of Brussels, died August 1 at the age of forty-seven years.

THE TOTAL APPROPRIATION for the U. S. Department of Agriculture for the year 1905 is $5,944,540. This includes $1,337,740 for the Weather Bureau.

DR. ROBERT BRAITHWAITE completed on his eighty-first birthday his monograph, the *British Moss Flora*, on which he has been engaged for twenty-five years.

EDWARD W. BERRY is studying the fossil flora of Maryland. His new address is in care of the Maryland Geological Survey, Johns Hopkins University, Baltimore.

THE NEW YORK Botanical Garden has purchased the entire mycological collections of Mr. GEORGE MASSEE, on which largely was based his work on *British Fungi*.

IT WOULD NOT be amiss for the editor of the *Zeitschrift für Pflanzenkrankheiten* to revise the list of collaborators on its title page. One at least has been dead these ten years.

WE LEARN from the *Journal of Botany* that Mr. GEORGE MURRAY has been compelled by failing health to resign his curatorship in the department of botany of the British Museum.

AN ADMIRABLE SUMMARY of the relation of plant physiology to the development of agriculture is presented by ALBERT F. WOODS, pathologist and physiologist of the Bureau of Plant Industry, in the Yearbook of the U. S. Department of Agriculture for 1904 (pp. 119–132), just issued.

THE ITALIAN Botanical Society devoted a special session at its recent general meeting in September to the memory of Professor F. DELPINO, of Naples, who died last May. The meeting was held in Vallombrosa, where DELPINO was formerly professor. A eulogy was pronounced by Professor BORZI, who was earlier his pupil and then his assistant.

PAUL PAREY (Berlin) announces that the third edition of the *Handbuch der Pflanzenkrankheiten* is in preparation by Professor Dr. P. SORAUER, who has associated with him Professor Dr. G. LINDAU and Dr. L. REH. They will treat respectively the plant and animal parasites which occasion diseases in plants, the original author, Dr. SORAUER, confining himself in this edition to the diseases due to conditions of weather, position, soil, and cultivation. The new work will appear in three volumes.